JOURNAL OF DAIRY SCIENCE

JULY, 1956

In this issue:

Abstracts of papers presented at fifty-first annual meeting

A review of calcium metabolism with special reference to parturient paresis

Association and industry news

Technical notes

Our Industry Today

Research articles on manufacturing and production topics

Abstracts of literature

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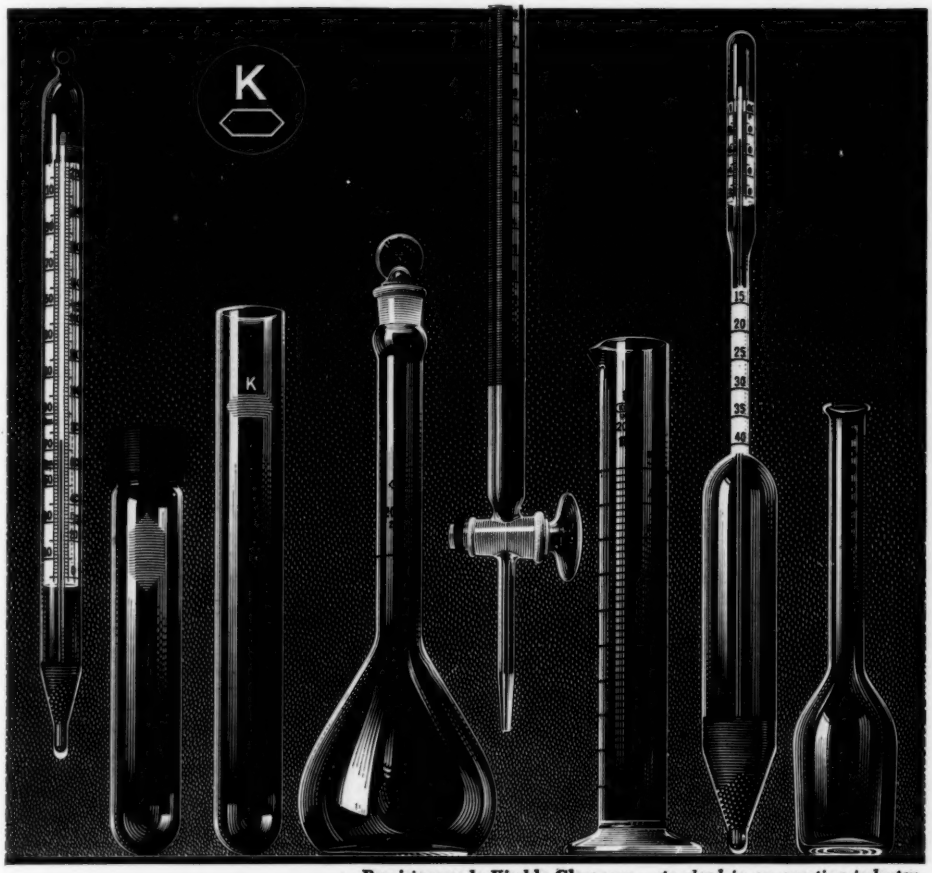

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PEOPLE and EVENTS
in the Dairy Science World

The Golden Jubilee Meeting

A total of 1,415 persons from 43 states, Washington, D. C., Alaska, and nine foreign countries gathered at Storrs, Conn., June 18-21 to exchange ideas on teaching and research, to visit friends, to enjoy the hospitality of the Connecticut hosts, and to properly celebrate the 50th birthday of the American Dairy Science Association.

Award winners recognized were as follows:

Teaching (Dairy Production)—W. E. PETERSEN, Minnesota

Extension Dairyman—J. O. TRETSVEN, Montana

American Feed Manufacturers Assoc.—J. B. SHAW, Maryland

American Dairy Science Assoc. Honorary Membership—H. F. JUDKINS

Borden, Manufacturing—E. O. HERREID, Illinois

Borden, Production—H. A. HERMAN, Missouri

New officers installed were:

President—C. F. HUFFMAN, Michigan

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Directors—E. H. PARFITT, Evaporated Milk Assoc., and G. M. WERNER, Wisconsin

Heard at the Storrs Meeting

W. E. PETERSEN (Univ. of Minn.) was given an honorary degree at the Univ. of Vermont June commencement.

ROGER W. HUNT has accepted a position with the A. & P. food laboratory as supervisor of all ice cream manufactured for the A. & P. food stores throughout the United States. He was formerly with the Sealtest Research Laboratory at Long Island.

J. B. FITCH, present head of the Dairy Dept. at Minnesota, is taking over the headship at the Univ. of Arizona September 1.

IRA D. PORTERFIELD assumed the duties of head of the Department at West Virginia Univ. July 1, replacing H. O. HENDERSON, who will continue his teaching and research activities.

F. V. KOSIKOWSKI of Cornell Univ. has been made an honorary member of the Veterinary Science Society of Italy for his contributions in the field of milk biochemistry and milk product technology.

A 62-page booklet on "Career Opportunities in Biology" by R. B. STEVENS, Executive Secretary, Biology Council NAS-NRC, has recently been released for distribution. Copies can be obtained for 48¢ each (less 25% school discount), plus postage from Row, Peterson and Co., 1911 Ridge Ave., Evanston, Ill.

Recent Deaths

W. H. HASKELL died in a Jacksonville, Florida, hospital on May 22 as the result of an operation for stomach cancer. He was born in 1889 at Taunton, Mass., and was graduated from the Univ. of Pennsylvania.

A veteran of World War I, he was associated with the U. S. Public Health Service for 21 years. In 1947 he became associated with Klenzade Products Co. as director of programs. During recent years he had been living in semi-retirement in Florida. He is survived by his widow and three children.

V. C. STEBNITZ, operator of the Chicago Dairy and Food Laboratory, died of a heart attack July 2 in Chicago. He was born at Arena, Wis., Dec. 2, 1900. He obtained a B.S. degree in 1931, a Master's degree in 1932, and a Ph.D. degree in 1935, all from the Univ. of Wisconsin. He was an assistant in dairy industry at Wisconsin from 1930 to 1937. From then until 1939 he was director of research for Beatrice Foods Company, and since 1939 he has been owner and director of the Chicago Dairy and Food Laboratory.

Higher Fellowship Awards for Student Dairy Judges

Increased DISA fellowship awards of $1,800, $1,650, and $1,500 for winners of the 1956 Collegiate Students' Intern. Contest in Judging Dairy Products, have been announced by Dairy Industries Supply Assoc., which with A.D.S.A. sponsors the annual tourney. The event will be held at Atlantic City, October 29, in conjunction with the 20th Dairy Industries Exposition. Fellowship increases this year—from the former $1,380, $1,280, and $1,180—are designed to attract high-caliber men with good undergraduate records. In addition to the fellowships, silver cups will be awarded to winning teams and wrist watches to individual first-place winners.

As in those of the preceding even-numbered years since World War II, the 1956 contest also will be a featured high light of the Exposition to be held at giant Convention Hall. The show will include displays of dairy industrial

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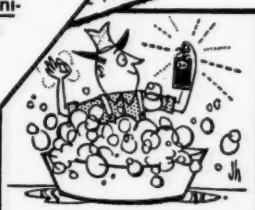

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Since the first contest in 1916 a total of 533 teams with approximately 1,680 contestants have taken part in the judging, in addition to about 430 alternate team members, who also have had an opportunity to sample products and to attend the expositions or conventions with which the event has been identified.

Dairy Parade to Be Seen in Chicago

Chicago's annual salute to the dairy farmer will be repeated in a great State Street parade October 6, the opening day of the 4th International Dairy Show. Sponsors are the *Chicago Daily News*, the Pure Milk Assoc., the American Dairy Assoc., and the Intern. Dairy Show.

The hour-long parade, featuring "Cows on State Street," will reflect all segments of the dairy world. Floats will show how good cows, nutritious feeds, sanitary processes, and quick transportation provide the nation with the most healthful food—milk and its products. Beauty and the beast will play an important part in the parade. ADA state princesses from the nation's leading dairy states will provide the beauty as they ride in open convertibles along the parade route.

Cows of the six dairy breeds—Ayrshire, Brown Swiss, Guernsey, Jersey, Holstein, and Milking Shorthorn—will show themselves off on specially designed tractor-drawn floats, decorated to portray the country of their origin.

Among the musical units in the parade will be the 5th Army Band, the famous Stock Yard Kilty band of bagpipers, and more than a score more of musical groups from throughout the Midwest. Cowboys and cowgirls astride western horses will be there from the world's championship Golden Spurs Rodeo, daily entertainment feature of the Intern. Dairy Show.

Dairy Science Bureau Changes Name

The official title of the Commonwealth Bureau of Dairy Science at Shinfield, Reading, has been changed as of May 1, 1956, to the Commonwealth Bureau of Dairy Science and Technology. This change is in conformity with recommendation 41 of the Commonwealth Agricultural Bureaux Review Conference Report.

Since its formation in 1938, the Bureau has been concerned with collecting published information on dairy research and issuing its journal *Dairy Science Abstracts*, first as a quarterly and since 1952 as a monthly periodical. Since 1951, also, this journal has contained review articles as well as abstracts. These have covered a wide range of subjects within the general scope of dairy science.

At the same time the Bureau has maintained an enquiry section which has undertaken to supply government officials, dairy research workers and advisory officers with specific information on given aspects of dairy science when asked for such information. This side of the Bureau's activities has grown considerably in recent years and is being increasingly used by the dairy industry, both in the U.K. and overseas.

Much of the work of the Bureau is, however, of a technological nature and intimately concerned with such subjects as dairy processing plant, dairy by-products, packaging and the materials of construction in dairies, as well as with the more scientific aspects of dairy research.

Career Day at California

A dairy industry career day was held on the Davis campus on May 5. About 60 high school seniors from throughout the state and a few junior college students, accompanied by University alumni and industry representatives, attended the meeting.

Laboratory demonstrations were set up in the Dairy Industry Building, and the University Creamery was in full operation. The schedule included a meeting with University officials, a noon luncheon sponsored by the California Creamery Operators Assoc., a guided campus tour, and an athletic program including swimming in the University pool.

A. F. TAMSMA, assistant specialist in the Department of Dairy Industry, has accepted a position with the Eastern Utilization Research Branch, USDA, in Washington, D. C.

E. N. FRANKEL, graduate assistant who has completed his graduate study, has accepted a position with the Northern Utilization Research Branch, USDA, at Peoria, Ill.

Touchberry to Study in Denmark

R. W. TOUCHBERRY, associate professor of dairy cattle genetics at the Univ. of Illinois, is leaving August 18 for Copenhagen, where he will study at the Royal Agriculture College with DR. KNUD ROTTENSTEN, an authority on the physiology of reproduction. Dr. Touchberry plans to visit Sweden, Scotland, Germany, and Switzerland before returning to this country next June. He will be accompanied by Mrs. Touchberry and their three children.

Ohio State News

Personal interviews and examinations of interested high school students have completed the 1956 dairy technology student recruitment program in Ohio. A total of 42 high school students competed for 9 available freshman scholarships. Examinations were held at Dayton, Toledo, Cleveland, and Columbus by boards including industry and department representatives. Schol-

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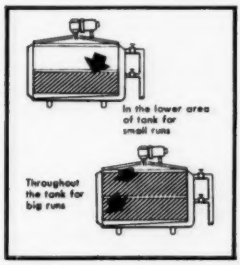

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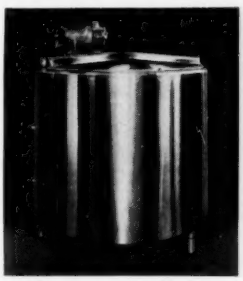

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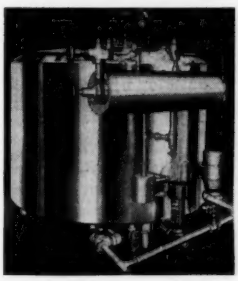

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arships were awarded on the basis of high school standing, interest in the dairy industry, and basic knowledge of English, mathematics, and science.

The scholarships are offered by the Maumee Valley Dairy Technology Society of Toledo, Northeastern Ohio Dairy Technology Society, Stauffer Dairy Technology Scholarship of Trumbull County, Beatrice of New Bremen, Columbus Milk Distributors Assoc., and the Dayton Milk Foundation. Two types of scholarships are available: $300 scholarships payable during the first year the student is enrolled at The Ohio State Univ. in dairy technology, and a $1,000 scholarship payable $250 each year for the 4 years a student is enrolled in dairy technology. Because of the large supply of well qualified applicants, an increased number of the $1,000 continuing-type scholarships is being encouraged.

Approximately 60 invited industry representatives, guests, and staff members attended a dairy technology research symposium on the Ohio State campus, May 15. The object of the symposium was to inform key men in the industry about the research being conducted in the department, application of research to practical problems, and an exchange of views in general.

The program opened with a luncheon in the Ohio State Union. N. P. HUDSON of the Dept. of Bacteriology delivered the keynote address, "The Philosophy of University Research." Market milk, concentrated milk, cheese, and cultures, and engineering and management were the fields covered in the symposium. Q. VAN WINKLE of the Department of Chemistry discussed the fundamental protein research. The remainder of the subject matter was presented by dairy technology staff members.

In line with the policy of the College of Agriculture to improve its educational program, the teaching staff of the Dept. of Dairy Technology has been holding weekly meetings at which time each course offered is being critically reviewed. Generally, about one-half day per week is devoted to the review of no more than two of the courses. The instructor in charge of the course presents the course program and other members join in analyzing the presentation and the effectiveness of the results. The objectives—*(a)* to improve the effectiveness of teaching, *(b)* to bring about an even greater amount of integration of the courses within the Department, *(c)* to look to possibilities of alteration in the curriculum.

The Governing Committee of the Ohio Dairy Products Research Fund held its annual meeting on the campus on May 15 to review the over-all research program in the Department of Dairy Technology. The main function of this committee is to review the work which is financed, at least in part, by the Ohio Dairy Products Research Fund. This fund was established at the University in 1947 by donations from members of the dairy industry and now totals approximately $129,500. This is deposited at 6% interest in the Ohio State Univ. Development Fund, and the interest is used for the continuing research program—mainly to support graduate students. The Governing Committee meets each spring and is comprised of the following members for 1956: Acting dean of the Graduate School, EVERETT WALTERS; dean of the College of Agriculture, L. L. RUMMELL; director of the Ohio State Univ. Development Fund, K. S. CAMPBELL; and representatives of the Ohio Dairy Products Assoc., who are ALLEN FRENCH, Cincinnati; J. H. ERB of Columbus; H. F. MEYER of Cleveland, with F. J. GREINER—executive secretary of the Ohio Dairy Products Assoc.—as ex officio. I. A. GOULD, chairman of the Dept. of Dairy Technology, serves as chairman of the committee.

A meeting of the joint Education Committee of Ohio Dairy Products Assoc. and Ohio Dairy Boosters was held in Columbus on May 24. Members of the committees are CARL BROUGHTON of Marietta, J. H. ERB of Columbus, W. C. HIMES of Dayton, LEWIS KOMMINSK of New Bremen, WAYNE BABCOCK of Toledo, MARK FULLER of Cincinnati, W. R. ISALY of Columbus, C. E. SCHROCK of Alliance, LESTER STAUFFER of Warren; and J. R. WIGGINS of Cleveland representing ODPA, and ED CLINGROW of Garfield Heights, HOMARD CLARY of Lakewood, and JERRY ZOELLER of Dublin representing the Boosters. At the meeting it was decided to revise the sequence of the slide production "Opportunities Unlimited" and make a record of the narrative part.

T. D. HARMAN spoke before fieldmen of the Evaporated Milk Assoc. in Columbus on April 2 and before the Indianapolis Dairy Technology Society on May 7.

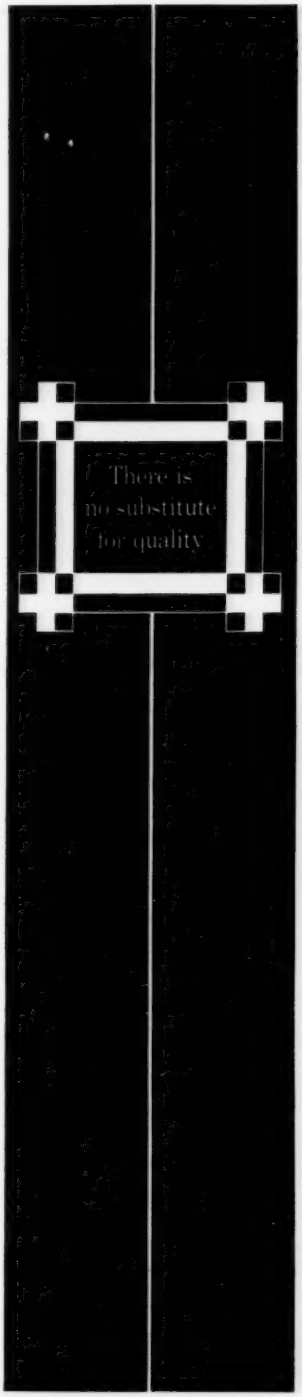
D. E. SEIBERLING spoke before the Assoc. of Ice Cream Manufacturers of Pennsylvania, New Jersey, and Delaware in Pocono Mannor, Pa., May 9, and before the Detroit Dairy Technology Society on May 14.

H. E. CALBERT, Dept. of Dairy and Food Industries, Univ. of Wisconsin, spoke before the Maumee Valley, Northeastern Ohio, Central Ohio, and Cincinnati Dairy Technology Societies on May 7, 8, 9, and 10. The topic presented was "New Developments in Quality Control of Fermented Dairy Products."

Increased effectiveness in teaching, research, and industry relationship will be the main topics on a Dairy Technology staff retreat to the Franz Stone Laboratories, Gibraltar Island, Lake Erie, in July.

H. P. Hood Purchases Orange Juice Plant

H. P. Hood and Sons, Boston, have purchased Minute Maid's concentrating plant at Dunedin, Florida, to be used for processing



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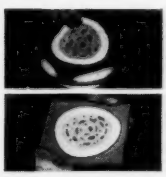

1. After filtering each can of milk (10 gallons or less), the producer carefully removes the used filter disk from the strainer and places it on a cardboard to dry.

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chilled fresh orange juice. This is in keeping with the trend in the dairy industry toward broad diversification of interests.

Hollender Receives New Assignment

Col. J. D. PETERMAN, commandant of the Quartermaster Food and Container Institute for the Armed Forces, Chicago, has announced the promotion of H. A. HOLLENDER to the position of associate director for research and radiation preservation of the Institute's food laboratories.

Since 1954 Hollender has been chief of the Dairy, Oil, and Fat Products Division of the Institute. In his new position he will have direct responsibility for the work of the chemistry and microbiology, food acceptance, nutrition, and radiation preservation branches of the Institute's food laboratories.

Upon completion of his undergraduate work at the Univ. of Wisconsin, Hollender received the 1940 and 1941 fellowship of the Dairy Industries Supply Assoc. and continued his studies at the Univ. of Illinois for his M.S. degree. From the fall of 1942 to the spring of 1943, he worked as a chemist for the War Department at the Badger Ordnance Works, Wisconsin. From May 1943 to February 1946, he served as a naval gunnery officer on merchant ships. After his war service, he returned to the Univ. of Wisconsin and received his Ph.D. degree in 1946. From 1948 until 1954 Dr. Hollender was associate professor in the Dairy Department of Purdue Univ.

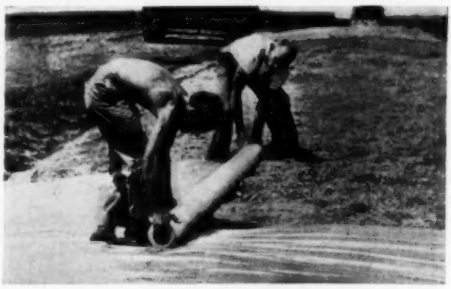
Polyethylene Roll Stock Used as Trench Silo Cover

According to the Continental Can Co., manufacturers of an all-weather polyethylene film, this product is being used by dairymen and beef raisers to cover self-feeding horizontal or trench silos. An unused roadway, an abandoned foundation, or areas beside an above-grade portion of a farm building make convenient sites for a trench silo. More elaborate wood and concrete-walled structures are prevalent as well. A common dimension for these silos is 20 by 80 ft., and, according to the depth of fill, they can contain up to 300 tons of ensilage. Chopped green fodder is blown into self-unloading wagons which, when full, discharge the feed into the trench. Ordinary farm tractors compact the silage into a firm mass simply by riding over it.

The polyethylene cover, in the form of sheeting 5 to 7 ft. wide, is rolled crosswise across the feed, with adjacent strips lapped 6 to 9 in. shinglewise to allow for water run-off. This width of sheeting is preferred by farmers because of ease of application and because it can be removed, sheet by sheet, and saved as the ensilage is required for feeding.

When the silo is located out in the open, and complete screening of the sun's rays is not possible, black polyethylene, which is impervious to ultra-violet light, is recommended. When enough matter can be spread over the film to screen out sunlight, uncolored or clear polyethylene has been found satisfactory.

Many feed lot operators, as well as dairymen, add self-feeding bunkers to the trench silos. The bunkers are moved back into the mass of the ensilage to enable the cattle to feed themselves with a minimum of labor on the part of the farmer.



Applying clear polyethylene film over chopped silage in an abandoned roadway trench silo site. Note that the film can be walked upon without damage.

Contact of air with silage results in oxidation of the fodder, rendering it black and unpalatable to cattle. In the absence of air, enzymes produce the desirable changes in the grasses, converting starches to sugars. The polyethylene strips, properly applied, not only seal off the ensilage from the air but also prevent wasteful leaching out of the nutritive sugars to the soil below. The film, applied directly from the roll, is weighted down with earth, chopped weeds, lime, or scrap lumber to keep it from blowing and to insure air-tight edges and laps.

Porter Joins Beacon Research Staff

The Beacon Milling Co. of Cayuga, N. Y., has announced the appointment of G. H. PORTER to their dairy research staff. Prior to his new appointment, Dr. Porter was an assistant in animal husbandry at the Univ. of Massachusetts and then an instructor in the Department of Dairy Husbandry at Penn State Univ., where he conducted research in calf nutrition. He was graduated from the Univ. of Massachusetts in 1949 and received his master's degree from the Univ. of Connecticut. At Penn State Dr. Porter coached the dairy cattle judging team and taught courses in dairy herd management, dairy cattle breeds and selection, and dairy cattle judging. He will headquarter at Cayuga and will reside with his wife and daughter in that area.

Seminar on Radioactive Milk at Cornell

JACK LUICK, Univ. of California, was the principal speaker in a discussion on radio-

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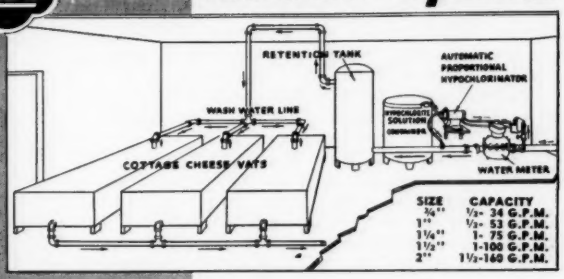

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active milk conducted by the Cornell Univ. Department of Dairy Industry, May 25. Material on the importance of isotopes in the dairy industry and the danger of radiation hazards was presented. Dr. Luick also demonstrated how milk labeled with radioactive Fe^{59} was prepared recently in his laboratory.

News from Maine

H. W. HALL, assistant professor of animal husbandry and assistant animal husbandman, Agricultural Experiment Station, retired July 1. He had served the University 33 years and was the author of a number of bulletins. His future plans have not been decided.

A new curriculum, dairy plant management, has been added to the list of majors offered by the Animal Industry Dept. This curriculum is broken down as follows: 25% basic sciences; 25% applied sciences; 25% business administration, and 25% miscellaneous requirements and electives. This program of study was developed to answer the growing need for students with business training for placement in managerial capacities.

Burrell Named NADEM Head

D. H. BURRELL, Cherry-Burrell Corp., was elected chairman of the Executive Committee of the National Assoc. of Dairy Equipment Manufacturers, at its 10th annual meeting, May 21-23, at Bethesda, Md. P. K. GIRTON, Girton Mfg. Co., was elected vice-chairman. Mr. Burrell succeeds E. M. HOWE.

Newly elected members of the committee are GORDON HOURAN of the DeLaval Separator Co., Poughkeepsie, N. Y., and GEORGE HUFFMAN of the Ex-Cell-O Corp., Detroit. Other members are E. M. HOWE, Waukesha Foundry Co. and HAROLD SOLIE, General Dairy Equipment, Inc.

EARLE SLAYTON, of the Cherry-Burrell Corp., who served as NADEM's first chairman when the organization was founded in 1946, made a major address, "Our First Ten Years," reviewing the past and pointing toward the future. C. M. FISTERE, General Counsel, spoke on the subjects of "Developments Along the Potomac" and "New and Pending Federal Legislation." O. G. SAXON, head of the Dept. of Economics at Yale Univ., spoke on "The Current Economic and Political Outlook." This was followed by a panel discussion on the stainless steel and nickel situation. The panel was led by D. C. BUCK of the U. S. Steel Corp.

News from Iowa State

J. M. RAKES has joined the Dairy Husbandry staff as a research associate. Rakes recently received the M.S. degree at the Univ. of Arkansas and now is working toward the Ph.D. degree in reproduction at Iowa State College.

The 6th annual cow conference is scheduled for September 7. This program is designed to acquaint dairymen in Iowa with new advances in production. Included will be a series of demonstrations on various aspects of research at the Dairy Husbandry Farm.

Creamery Package Promotes Bell

According to a recent announcement from J. L. BRAZEE, vice-president of the Creamery Package Mfg. Co., Chicago, manufacturers of dairy processing and refrigerating machinery, R. D. BELL has been promoted to sales manager of the firm's Commercial Division. In between serving with the U. S. Army in Europe and a second hitch in Korea, Mr. Bell earned a degree in chemical engineering at the Univ. of Illinois. Before joining Creamery Package, he was a project engineer for the Department of Defense, U. S. Government.

Babcock Promoted in USDA

C. J. BABCOCK has been made director of the Dairy and Poultry Division of the Foreign Agricultural Service of the U. S. Department of Agriculture. His former position as chief of the Foreign Marketing Branch of the Dairy and Poultry Division has been filled by D. R. STROBEL.

Completed Theses

M.S. Degree:

ANTHON H. CUMMINGS—The effect of feeding high levels of chlortetracycline (aureomycin) and tetracycline (achromycin) to new-born calves. Louisiana State Univ., Baton Rouge.

BARNEY HARRIS, JR.—The effect of aureomycin and diethylstilbestrol upon growth, health, and endocrine organs of dairy calves when fed singly and in combination. Louisiana State Univ., Baton Rouge.

HOWARD C. KELLGREN—The response of Holstein-Friesian, Jersey, Red Sindhi–Holstein (F_1) and Red Sindhi–Jersey (F_1) bulls to a simulated subtropical climate. Louisiana State Univ., Baton Rouge.

CLYDE L. LEWIS—The influence of season of freshening on milk production of Jersey and Holstein cows in Louisiana. Louisiana State Univ., Baton Rouge.

FRED B. MCDANIEL—The influence of acidity control during the manufacturing process on the market quality of cottage cheese. Oregon State College, Corvallis.

I. SATO—Some factors affecting the freezing point of milk. The Ohio State University, Columbus.

KENNETH W. TWIGG—Sherbet studies using orange and cranberry concentrates. Oregon State College, Corvallis.

Master of Agriculture:

WILLIAM L. NORTHERN—(Completed problem) The correlation between the quality of roughage fed and amount of butterfat produced. West Virginia Univ., Morgantown.

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Ph.D. Degree:

JOHN V. BATEMAN—Some chemical and bacteriological changes, nutrient losses, digestibility, and productive value of grass silages made with and without preservatives. Oregon State College, Corvallis.

CLARENCE CHESNUTT, JR.—The nutritive value for dairy cattle of irrigated pasture herbage under normal rotational grazing, strip grazing and green soiling methods as measured by digestibility studies and animal performance. Oregon State College, Corvallis.

K. K. FOX—The effect of heat on the electrophoretic properties of milk proteins. The Ohio State University, Columbus.

PREM NATH NARULA—The distribution of nitrogen compounds in goat's milk. Oregon State College, Corvallis.

RICHARD D. PARKER—Nutritional factors in milk affecting growth of lactic acid streptococci and lactobacilli. Oregon State College, Corvallis.

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The ABSTRACTS are issued quarterly by the Commonwealth Bureau of Animal Breeding, Edinburgh, at 50s. ($7.00) per annum. Subscriptions and enquiries should be sent to the address below or may be placed with any subscription agent.

* In view of numerous requests, a reprint has been issued of the review article in Vol. 23, No. 3 (1955) by D. H. L. Rollinson, "Hereditary factors affecting reproductive efficiency in cattle," priced 2s, 6d. ($0.35).

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STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

What Industry Expects of the College Graduate

Qualifications important for the success of individuals in industry were recently discussed by W. E. GLENNON, president of the American Feed Manufacturers Assoc., before the Teaching Section of the American Society of Animal Production. The following excerpts from Mr. Glennon's talk are "bits of wisdom" for student, teacher, and current industry employee.

"The future growth and achievements of our industry are going to depend on the men who will be tomorrow's leaders. A company or industry can be no better than the individuals who comprise it. Human engineering is still the most important aspect of any business. An industry does not stand still. It either grows or dies.

"There will be an increasing demand for well-trained young men in our industry.

"General qualifications important for the success of a person in any industry whether he be a graduate of agriculture, engineering, liberal arts, commerce, or whatever the course, include dependability, loyalty, ambition, initiative, and imagination. He should have a pleasing personality and the desire and ability to get along with his fellow men and to fit into an existing organization, personnel, or situation. He may have the finest training and a superior I.Q., but faulty personality traits will be a definite handicap. Let me hasten to point out that perhaps in certain industries and large companies where this type of man has a definite assignment in a large development and research department, and his activities are limited to work on one subject or single project on which he may be highly qualified, he could be eminently successful.

"The industry expects and will require that the candidate for employment have the ability and desire to continue to study and learn. At the rate of speed that nutritional discoveries and applications are being made today, this is absolutely essential. Certainly there is no need to elaborate on this point. Try to impress on them that, despite the prevalent philosophy of the day, they will be wise to put less emphasis on future security and more emphasis on future possibilities.

"One weakness that seems to be almost universal in young graduates and in all of us, for that matter, is the inability to express ideas clearly and forcefully in either oral or written form. By this we do not mean that it is necessary to be a polished speaker, but some training in public speaking is very desirable. Likewise, the ability to write in a precise, interesting, forceful, and informative manner can help tremendously. Encourage your students to give brief reports orally in front of the entire class as frequently as possible. They may not like it —but it's wonderful experience.

"At least a little knowledge of such subjects as business letter writing, literature, art, and perhaps some of the other cultural subjects is worth while. Encourage them to participate actively in some activities such as clubs and other groups that are common on every college campus.

"Tomorrow's successful man won't be a 'know-it-all' but, rather, one who knows 'where to go to', 'how to work with' purchasing, production, sales, advertising, and 'how to get' advice, weigh the facts and move ahead with adjustments—enthusiasm—foresight—optimism—and purpose."

Florida Dairy Science Club Sponsors Pasture Exhibit

The Dairy Science Club exhibit at the Agriculture Fair, sponsored by Alpha Zeta, had the dairy producer in mind. The theme was "Better Pastures for Greater Profits."

The Agriculture Fair Committee consisted of DICK HOLTSCLAW, chairman; CHARLIE DUNNIGAN, and CHARLIE BECKER. All the work was done by club members. The object was to give a planned yearly pasture program and show how better pastures give greater profits by reduction of feed costs. It showed three pastures —excellent, fair, and poor—the amount of supplement needed to produce 30 lb. of 4.0% milk, the cost of each, and the total feed cost for the milk. Literature was handed out which gave more detailed information. The theme of the exhibit was well presented and it added tremendously to the success of the Fair.

On March 9, the Dairy Science Club held election of officers. DOUG BOYETTE of Hollywood is the new president; JAMES THORNHILL of Winter Haven, vice-president; KAREN BERLS of St. Augustine, secretary and treasurer; ROBERT MOSELEY of Winter Park, reporter. H. H. WILKOWSKE is faculty advisor.

Little American Royal of Kansas State College

The 28th annual Little American Royal was held April 7, in Ahearn Fieldhouse, on the Kansas State College campus. This show was started in 1924 as entertainment for Farm and Home Week visitors. It blossomed into the biggest event of the school year for K-State Ag. students. Originally started as an afternoon

event, its wide popularity resulted in its expansion to an evening program with admission by tickets necessary to assure guests of seats.

Because of a small student enrollment during World War II, the Little American Royal was discontinued after 1942 but was reinstated in 1948 under the cosponsorship of the Dairy and "Block and Bridle" clubs.

Judges place equal emphasis on fitting and showing. However, consideration is given to the improvement a student has made on an animal during the 7-week fitting and showing period. The basis for judging improvement is the difference between the grade given the animals before the fitting period begins, and the grade given just before the show.

This year more than 175 students competed. ANCEL ARMSTRONG was selected grand champion showman, showing a Jersey heifer. Last year Ancel won the Ayrshire breed championship. The year previous he won second in the Jersey heifer class.

Kentucky Club Holds Dairy Career Day

The Univ. of Kentucky Dairy Club held its annual Dairy Career Day on April 27. This event is held each spring in an effort to acquaint graduating high school seniors with the University, the Dairy Club, and Dairy Section. Sixty-six high school seniors, sponsored by local dairy organizations, attended this activity. The boys were given tours of the campus and the experiment farm by members of the Dairy Club and the dairy faculty.

An Honor Banquet was given at which an outstanding man in the dairy industry was honored. The person receiving the honor this year was JOHN MOSER of Louisville, Ky. Awards and prizes were also given to the winners of the spring judging contest. This contest is held each year and consists of both cattle and dairy products judging. Anyone enrolled at the University is eligible to participate. A great deal of interest was shown in the contest this spring with a total of 52 participating. The winners of the cattle contest were: upper division cattle, SMITH MITCHELL; lower division, CLYDE ALLEN. The products winners were: all products, EDWARD C. PRICE; cheese, JOE LAMPKIN; butter, JOHN BOSTON; ice cream, DICKIE FEATHERSON; and milk, BILL SOARDS.

LSU Dairy Science Club Activities

The LSU Dairy Science Club is finishing the year with a surge of activities. The annual student judging contest, held April 7 with several classes of dairy cattle and products, was very successful, and the barbecue which followed it was enjoyed by all. A likely prospect for the judging team was turned up when WILLIAM HIGHTOWER, a dairy production major, walked off with high scores in cattle placing and reasons and also in products judging.

The Club cooperated with the Block and Bridle Club in putting on the "Little International," March 26. Club members won several awards in this contest.

KENNETH CONLY, a Dairy Club member, was selected as one of the two southern regional winners of the Sears-Roebuck Foundation Scholarships. He received an all-expenses-paid trip to Chicago, where the final national winners were selected.

The Club has also put on district and state judging contests for high school FFA and 4-H teams. The Club members obtain valuable experience in organizing and putting on these contests and also help to stimulate interest in dairy cattle among the juniors who will be our future dairymen.

The Club's annual banquet and dance was held on May 5. The program was highlighted by the naming of the "Dairyman of the Year." This honor was conferred upon C. C. SCHOUEST, owner of the Sterling Ice Cream Co. of New Orleans. Mr. Schouest is a club alumnus and has been very active in promoting the growth of the dairy industry in Louisiana. Also honored for their contributions to the dairy industry were: the late J. G. LEE, JR., dean of the College of Agriculture for 25 years; DAVE PIERCE, State Commissioner of Agriculture and long-time supporter of the dairy industry; and B. B. JONES, Agricultural Extension Service, retired. Honorary club memberships were conferred on these men in recognition of their long service to agriculture and the dairy industry. The banquet was followed by a dance which completed the year's activities of the Club.

Minnesota Dairy Science Club News

Miss Evelyn Jackman at Minnesota "Milk Hour"

Production majors of the Minnesota Dairy Science Club journeyed to Rochester, Minn., on April 7 to attend the Black and White Dairy Cattle Show. This has become an outstanding

spring show as a result of enthusiastic support evidenced by the high quality of dairy cattle exhibited. Club members participated in the annual cattle judging contest with DALE BLANK, Club president, placing first, and DONALD OTTERNESS, former Club secretary, placing second in the open-class contest.

Over 750 students and faculty were guests of the Dairy Science Club at the winter and spring "Milk Hours" held at the Student Union on the St. Paul campus. This has become the most popular activity of the club and will be continued on a quarterly basis. Assisting with the spring quarter Milk Hour was EVELYN JACKMAN, first attendant to Minnesota's "Princess Kay of the Milky Way." She is currently a student in home economics at the University.

Club members assisted with open-house tours in the Dairy Department during the Minnesota Royal, an annual 3-day event sponsored by all agricultural students on the St. Paul Campus. The Royal was well attended by friends and parents of students and also by over 1,000 FFA members concurrently attending their state convention on the campus. A popular feature of the dairy open-house was the serving of free ice cream to visitors by "Princess Kay," RUTH MARIE PETERSON, and her first attendant, EVELYN JACKMAN.

Dairy majors were active in judging activities during the Royal, participating in the showmanship contests and also assisting with the FFA dairy judging contests.

The year's activities closed with a Club-sponsored student-faculty "steak-fry," held May 24.

Montana Dairy Science Club Spring Quarter Activities

The members of the Montana Chapter have had much activity during the spring quarter, both as a group in the chapter, and as individuals working in other school activities.

In April, the club voted to donate $5 toward the cost of putting an identification sign on the Agricultural Building. This project is being carried on in cooperation with the other clubs which meet in the Agricultural Building.

The club sponsored a picnic in May and invited the faculty, barn laborers, and special guests. A hot game of softball was played until dark, and then everyone went inside for swimming and roller skating.

The old saying, "The third time is the charm," came true for one of our members, NORVAL MARSH, this year. Norval was chosen Grand Champion Showman at the college Little International Livestock Show. Norval has been in the final ring the past 2 years, but this year he came out on top.

In conjunction with the Little International, the club put up a dairy display showing the processing of milk from the cow until it goes into the bottle. Many favorable comments were received about this display.

It has been a very successful year for the club, because of the greater activity and closer feeling of kinship generated among the members and faculty.

Oklahoma A. & M. Dairy Science Club News

Highlighting the program of the Dairy Science Club banquet held April 28 in the Student Union was the presentation of the outstanding senior awards by C. L. NORTON, Dairy Department head. CHARLES YOUNG, Billings, was named the outstanding senior majoring in dairy production. MACK TURLEY, Anadarko, was the winner of the A. C. Baer award for the outstanding senior majoring in dairy manufacturing. Other awards were presented to the winners of the freshman-sophomore dairy cattle and dairy products contests, which are held each year in cooperation with Alpha Zeta.

This year the club established a "Good Indian" award honoring club members who had worked hard for the club, but who in the past received little credit for their efforts. A Good Indian was selected for each club project and the Good Indian of the year was in turn selected from them. MACK TURLEY was named the all-round Good Indian for the 1955-56 school year.

On May 11, all students in the School of Agriculture gathered at Camp Redlands for the annual Aggie Day. Each year an Aggie Princess is selected from the queens of the various agriculture departmental clubs. This year the Dairy Science Club's queen, MISS KAY STRAIN, topped the class and walked away with the royal honors. Kay will be entered in the State Dairy Princess contest later this year.

The club's softball team also made a good showing on Aggie Day by placing second in the departmental club tournament.

JOHN EGERMEIER, a junior in dairy production, received the Ralston-Purina award for the outstanding junior studying in the field of agricultural nutrition. He will be awarded $500 to apply toward further study in the nutrition field.

On May 18, Fair Park was the scene of the annual Dairy Department picnic. Main event of the day was the yearly struggle between the dairy faculty and the club members, which may be broadly termed a softball game. As usual, both sides boasted outstanding teams.

V.P.I. Seniors Honored

W. B. ALLISON, JR., a senior in dairy science at V.P.I., received the Alpha Zeta Outstanding Senior Award for 1955-56. This award is based on scholastic achievement, leadership ability, character, and extra-curricular activities. During the past year he was director of the Agricultural Exposition, business manager of the Tech-Nique, and president of the Virginia Tech Chapter of A.D.S.A.

ALLEN H. RAKES, a senior in dairy science, was the recipient of a Ralston-Purina Graduate Award in Dairy Science. He will pursue gradu-

ate work in the Dept. of Dairy Science at V.P.I. Rakes was also the recipient of the Borden Foundation Award.

Washington State Chapter Sponsors Banquet

The W.S.C. student chapter of A.D.S.A. sponsored the 25th annual banquet of the Institute of Dairying on the evening of March 13. Approximately 190 dairy operators and guests from Canada, Alaska, and the U. S. took part in the Institute this year. General chairman for the banquet was JOHN WILLEMSON, and JIM CALVERT served as toastmaster. The theme of the banquet was the celebration of the silver anniversary of the Institute. Entertainment was furnished by the "Chucklers," an all-college western entertainment group. CHARLOTTE MCQUEEN, the 1955 Washington State Dairy Queen, presented a humorous act. The final event of the evening was the showing of a series of slides which gave guests a look at the agricultural program in Pakistan. S. P. SWENSON, dean of the College of Agriculture at W.S.C., presented them along with comments about his trip to that country.

Four honorary "Doctor of Institute of Dairying" degrees honoring the guests of the Institute were presented. Recipients were: H. C. OLSEN, Oklahoma A. & M. College; D. V. JOSEPHSON, Pennsylvania State Univ.; E. B. KELLOGG, secretary, Milk Industry Foundation, and G. S. BULKLEY, Carnation Co. These men were largely responsible for the success of the Institute.

LETTERS TO THE EDITOR

President I. A. Gould has made a stirring appeal for support of the American Dairy Science Association by reading and subscribing to its publication. (Journal of Dairy Science **39**: 19, April, 1956). "It seems logical that every graduate from a dairy school would feel a sense of obligation and loyalty to his professional organization and would want to contribute his small bit to help make certain that the group remains strong and influential. This he should accept as an obligation, whether or not he reads the Journal and whether or not it is, in his opinion, of any specific benefit to him. This is, in effect, the principle of supporting a 'just cause'—where the benefits to the individual may not always be clear—but where the support makes possible the continuation of a worth-while industry program."

In the December issue (Journal of Dairy Science **38**: 1412. 1955) there appears a Call for Papers for the Annual Meeting. "Reactions of those attending previous meetings indicate that papers presented by members in industry and by senior members of the Association are especially well received." Does this statement reflect a general philosophy of the organization toward presentation of papers by graduate students? It appears to be inconsistent with the request of President Gould. How does one become a loyal senior member of the society if it is not by participation in its activities? Certainly, the conduct of an Annual Meeting is one of the most important functions of a scientific society. A mature association encourages participation in this activity of the society by presentation of papers at the Annual Meeting. Scientific merit of the subject matter rather than age or experience of the contributors might be the principal basis for suitability of the paper, although much can be learned from a philosophical and informational viewpoint from senior members of the society. Encouraging participation by junior members can certainly be expected to build a strong society of senior members. It is difficult to ask for subscription and reading support if a more active participation is not encouraged. The first step might be simply to delete from the Annual Call for Papers the statement which discourages participation by graduate students. A suggestion might be made that stimulating and scientifically sound papers presented by graduate students are enthusiastically received.

R. G. HANSEN
Professor
Dairy Biological Chemistry
AND
DON M. CARLSON
Graduate Student
Dairy Biological Chemistry
Univ. of Illinois

Owing to some confusion in mailing the January and February 1956 issues, some persons received duplicate copies of the Journal. If anyone now having duplicate copies for either or both of these months will return them to The Garrard Press, 119 West Park Avenue, Champaign, Ill., the postage charge will be refunded. This will help relieve an acute shortage. Thank you.

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University of Connecticut

Storrs, Connecticut

June 19-21, 1956

MANUFACTURING SECTION

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M1. Characterization of phosphorus compounds in alcohol-ether extracts of milk. ALAN KENYON and ROBERT JENNESS, Univ. of Minnesota, St. Paul.

Attempts have been made to identify phosphorus compounds that are co-extracted with the fats and phospholipids of milk by the Bloor alcohol-ether technique, which consistently extracts about 5 times as much phosphorus as the Roese-Gottlieb method. The difference is not due to inorganic phosphate, because P^{32} added to milk in the form of potassium phosphate was not detected in the Bloor extract, and inorganic phosphate was not detected in the extract by ferrous sulfate and molybdate reagent. Lipids were removed from the Bloor extract by reextraction with chloroform, leaving a water soluble residue that contained the nonlipid phosphorus compounds. A classical barium fractionation of this residue indicated the presence of a small amount of sugar phosphate esters in the barium-soluble, alcohol-insoluble fraction, but most of the organic phosphorus remained in the barium-soluble, alcohol-soluble fraction. This material appears to be unionized since a spot of it on paper at pH 8.6 did not migrate when an electrical potential was applied and since it was not retained on an anion exchange resin. Finally, the compound or compounds are only slowly hydrolyzed by acid to yield inorganic phosphate.

M2. Preparation and characterization of two fat-globule membrane fractions from normal milk. C. T. HERALD and J. R. BRUNNER, Michigan State Univ., East Lansing.

Recent studies in this laboratory indicate that the most tenacious membrane proteins can be resolved into soluble and insoluble fractions. Membrane-containing serum was obtained from washed cream prepared according to classical methods. The membrane proteins were concentrated by salting out with 55% ammonium sulfate and subsequent centrifugation (25,000 G $\times$ 30 min.). Lipids were removed with cold ethanol-ether treatments followed by several ether extractions at 30° C. Repeated extractions with 0.02 *M* sodium chloride were utilized to separate the protein into fractions. The soluble fraction gave a strong Molisch reaction, reduced Fehling's solution subsequent to mild acid hydrolysis, was sulfhydryl-negative after heating to 75° C., and had nitrogen values varying with preparations from 9.5 to 11.5%.

The residual fraction was insoluble in 25% sulfuric acid. However, sodium sulfide and Duponol PC are representative of reagents capable of solubilizing this material. Nitrogen values ranging from 12.9 to 13.9% were observed. A qualitative sulfhydryl-positive reaction was obtained upon flash heating the insoluble protein moiety to 75° C.

M3. Relation between loss in solubility to a fat-protein complex formation. I. I. LITMAN, U. S. ASHWORTH, and H. A. BENDIXEN, State College of Washington, Pullman.

Evidence was obtained for the development of a fat-protein complex in whole milk powder which contributes to loss in solubility. In reconstituted milk powder, formation of "scum," as insoluble material clinging to the walls of the container, occurred only with powders containing fat, and increased during storage at room temperature and above. Storage at 45° F. effectively retarded its development. Although the free-fat content of the milk powders diminished rapidly during storage at 85° F., it did not diminish at 45° F. The free-fat content may, therefore, be related to scum development.

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The scum contained an average of 48% fat, which had a higher melting point and a lower iodine value than the original fat in the powder. It contained 34% protein, independent of powder composition. The protein fraction was found to be extremely unstable by the alcohol stability test. It consisted of 82 to 96% casein plus denatured whey protein, which was related to the preheat treatment of the original milk. Scum solids contained a greater amount of calcium than the remaining solids.

M4. Hydrogenation of butter oil. H. D. WEIHE, Eastern Utilization Research Branch, USDA, Washington, D. C.

Butter oil, in 400–500 g. batches, has been hydrogenated by using 0.5% Raney nickel catalyst under 1 to 2 atmospheres of pressure. Temperatures were 125° and 150° C., with at least 3 degrees of hydrogenation at each temperature; the most extensive hydrogenation was to an iodine value of 20.9. After the filtering off of the catalyst, the hydrogenated oil was heated at 100° C. for 1 hr. at about 5-mm. pressure with nitrogen gas bubbling through for agitation and removal of all hydrogenation odors. Some butter aroma remained with moderate degrees of hydrogenation. Recombined homogenized whole milks, made from these hydrogenated butter oils and fresh skimmilks, were stored at –17° C. in small sealed cans. For the most part, flavors were rated acceptable for at least 134 days, whereas controls were unsatisfactory after 1 mo. Less hydrogenation was required at 150° than at 125° C. for the same degree of flavor stability. In another series of experiments 1,300-g. batches were hydrogenated at 150° C. and 4.4 atmospheres pressure. Hydrogenated deodorized butter oils showed increased stability, based on the number of days in storage at 55° C. required to reach a peroxide value of 1, with increase in degree of hydrogenation, stability being six times the control for a sample hydrogenated to an iodine value of 20.6.

M5. Application of gas-liquid partition chromatography to the study of volatile flavor compounds. W. G. JENNINGS, Univ. of California, Davis.

Gas-liquid partition chromatography offers a versatile new technique which simplifies the separation and analysis of volatile mixtures. The method shows promise for the study of natural flavor compounds because of the small amounts of material that can be handled and the tremendous resolution that is achieved. The process resembles chromatography, with the eluting liquid replaced by a carrier gas. The sample is not adsorbed to a surface but dissolved in a nonvolatile oil distributed on a finely divided inert solid.

The method is here applied in a study of the volatile compounds present in commercial starter distillate. The selection of starter distillate as a starting material was influenced both by its availability and by the desirability of establishing a correlation between specific chemical compounds and flavor characteristics in cultured dairy products.

The distillate was concentrated in a modified Oldershaw column and the concentrate was extracted with ethyl ether. The extract was dried over $MgSO_4$ and most of the ether was removed by fractional distillation. The concentrated volatile fraction so obtained was subjected to gas-liquid partition chromatography. The component fractions were characterized by methods that included spectrophotometry, formation of derivatives, and comparison of R_f values.

M6. Colorimetric determination of fat in milk by the hydroxamic acid reaction. RICHARD BASSETTE and MARK KEENEY, Univ. of Maryland, College Park.

A procedure has been developed by which micro-quantities of milk are analyzed for fat content by the hydroxamic acid reaction. Milk is reacted with alkaline hydroxylamine in isopropanol. The reaction mixture is diluted with acidic methanol containing ferric chloride. The red color intensity of the ferric hydroxamate solution is measured in a photoelectric colorimeter equipped with a 525-mμ filter. The fat content of the milk is calculated from the color intensity by referring to a standard curve.

M7. Separation of the major components of the milk lipase system by supercentrifugation.[1] W. J. HARPER, I. A. GOULD, and M. BADAMI, The Ohio State Univ., Columbus, and Ohio Agricultural Expt. Sta., Wooster.

Freeze-dried raw skimmilk powder was reconstituted at various pH levels and centrifuged at 25,000 G and 50,000 G. The distribution of the 2 major components of the milk lipase system between the sediment and the supernatant layers was determined by measuring the lipase activity at their respective pH optima (7.0 and 8.6). (Two major components were reported by Schwartz *et al.*, 1954.)

Centrifugation of milk reconstituted at pH 7.0 revealed that the 2 lipases were completely separated. All of lipase A (pH optimum 8.6) was found in the sediment layer, whereas all of lipase B (pH optimum 7.0) was found in the supernatant layer. Centrifugation at pH values of 6.0 and 8.0 resulted in different distribution of the 2 lipases between the sediment and the supernatant layers.

These results indicate that lipase A was associated with the casein and could be centrifuged with it. The degree of association could be varied by altering the pH of centrifugation. Data also showed that sedimentation of lipase A removed an inhibitor present in the milk serum.

[1] Supported in part by the Ohio Dairy Products Research Fund and from Ohio University Research Foundation Grants for Fundamental Research.

M8. The water-insoluble acid content of fresh milk. T. R. FREEMAN and W. F. LEWALLEN, Kentucky Agr. Expt. Sta., Lexington.

Individual cow samples of milk were obtained once a month at the morning milking from all producing animals in the Experiment Station herd. Immediately after collection these samples were pasteurized at 160° F. for 30 min., cooled to 95° F., and separated with a mechanical separator. Water-insoluble acid (WIA) values were then obtained on the cream by Hillig's rapid method. WIA values were obtained within 1.5 to 2 hr. after milking.

In another series of trials, half of each individual cow sample of milk was treated as outlined above. The other half was separated raw and the resulting cream was stored at 50° F. for 24 and 48 hr. prior to WIA analysis.

Highest WIA value was 122, lowest 31, and average 67, from 237 samples of Jersey milk. Corresponding values from 247 samples of Holstein milk were 130, 33, and 74, respectively. Highest average values were obtained in June (87) and lowest in July (53). Stage of lactation did not affect WIA values.

Storage at 50° F. increased the WIA content of Jersey cream 158% in 24 hr. and 164% in 48 hr. For Holstein cream the values were 344% and 377%, respectively. The least increase occurred in September and October.

M9. Effect of time of lactation and of summer and winter feeding on the development of rancidity in milk. E. S. GUTHRIE and B. L. HERRINGTON, Cornell Univ., Ithaca, N. Y.

There is a difference of opinion among dairymen as to the importance of milk from stripper cows as a cause of rancidity in milk supplies. To secure more information on this point, the milks from 36 individual cows have been held at 35-40° F. for 24 hr. and then tested for rancidity by analysis for free fatty acids and by organoleptic examination. These tests are being repeated at regular intervals until the cows go dry, to observe any changes in lypolytic activity that may occur with advancing lactation and drying off.

M10. Changes in the composition of the proteins present in milk during the lactation period. B. L. LARSON, G. D. ROLLERI, and K. A. KENDALL, Univ. of Illinois, Urbana.

Studies were made of the specific proteins present in the milk of 8 cows during their lactation periods. The amount (g. per 100 ml.) of both total casein and the serum proteins in the milk decreased from about 6 g. and 8 g., respectively, at parturition to 2.5 g. and 0.5 g. at the end of the first month, and then increased to 3 g. and 0.8 g. at the end of lactation. A good many breed and individual differences were noted.

Normal percentages of α-, β-, and γ-casein in the total casein were about 66, 29, and 5, respectively. The percentage of α-casein remained fairly constant. γ-Casein was especially high and β-casein low just after parturition. The percentage of γ-casein tended to increase in late lactation at the expense of β-casein (up to 15% for some cows).

The percentages of α-lactalbumin and β-lactoglobulin in the serum proteins increased for the first month after parturition, remained rather constant for each cow (22% and 58%, respectively), and then tended to decrease in late lactation. The immune euglobulin and pseudoglobulin fractions decreased from 75% to 13% during the first month and then increased up to 25% at the end of lactation. Serum albumin (about 5%) fluctuated and showed no characteristic trends.

M11. The relation of milk serum proteins to the effects of heat treatment on rennet clotting. A. KANNAN and ROBERT JENNESS, Univ. of Minnesota, St. Paul.

The possible involvement of the milk serum proteins in the prolongation of rennet clotting by heat treatment was investigated by means of simplified systems. Caseinate was separated from skimmilk by high speed centrifugation and was then dispersed in a dialysate prepared by equilibrating a small amount of water against a large amount of skimmilk. Unheated sols of this kind had rennet-coagulation times comparable to those of skimmilk. However, heating at 85° C. for 30 min. did not increase the coagulation time as it does for skimmilk. Inclusion of freeze-dried milk serum proteins in the sols restored the response to heat treatment. The increase in rennet-clotting time produced by heating became greater as the concentration of serum proteins was raised. The effect was observed only when the caseinate and serum proteins were heated together. Separate heating of either or both did not produce an increase in rennet-clotting time.

M12. Physical and chemical properties of some high molecular weight complexes isolated from heated skimmilk. M. J. PALLANSCH, H. S. HALLER, and A. G. MORIN, Eastern Utilization Research Branch, USDA, Washington, D. C.

The effect of heating on the individual caseins found in the protein micelles dispersed in milk was studied by electrophoretic and ultracentrifugal methods. Samples of milk heated at 115° C. for varying periods of time were centrifuged to isolate dispersed colloidal phase before analysis. Results obtained indicate that a new electrophoretically unique compound appears in the high molecular weight fraction of milk when it is heated. This new material has a mobility between that of α-casein and β-casein. The amount of the material present increases with increasing heat treatment. It was also found that the sedimentation constant of the α-casein isolated from heated milk was considerably lower than that of comparable material isolated from unheated milk.

Concentration of milk before heat treatment increased the amount of heat-induced change observed in the electrophoretic patterns.

Results of heating experiments carried out with purified α-casein and lactose are interpreted to indicate that a lactose-protein interaction is responsible for some of the observed changes in the electrophoretic mobility and may possibly be related to some observed changes in sedimentation constants that occur in the proteins of the disperse phase when milk is heated.

M13. Electrophoretic and sedimentation characteristics of two fat-globule membrane fractions from normal milk. J. R. BRUNNER and C. T. HERALD, Michigan State Univ., East Lansing.

The membrane proteins were resolved into 2 fractions based upon solubility in 0.02 *M* sodium chloride solution. The soluble fraction was equilibrated and examined electrophoretically in the following buffer systems of 0.1 ionic strength: veronal, pH 8.7; phosphate, 7.3 and 6.1; acetate, 5.3 and 4.3; glycine-HCl, 3.8 and 2.1. The pH values reported are at 1° C. Electrophoretic patterns showed a 2-component system of nearly similar mobilities. Low component mobilities were recorded at pH 4.3 and 6.1. A portion of the soluble fraction was precipitated during dialysis at pH 5.3, and inappreciable component mobilities were observed in the supernatant.

The insoluble fraction was solubilized with sodium sulfide and also with Duponol PC. Whenever applicable, the buffer systems previously described were employed in the electrophoretic studies. Electrophoretic patterns of the sodium sulfide–treated samples showed a sharp, almost symmetrical peak. The patterns for samples solubilized with Duponol PC indicated a 2-component system.

Sedimentation diagrams of the soluble membrane-protein fraction in veronal-citrate buffer at pH 8.0, ionic strength 0.09, showed a single, broad-component peak.

M14. Magnesium in the casein-containing colloid of milk. T. G. ALEXANDER, Eastern Utilization Research Branch, USDA, Washington, D. C.

Magnesium exists in milk as a part of the colloid system and in solution. A study was made on the proportion bound to the colloid and the condition of this magnesium. The colloid was separated by centrifugation and was washed free of serum. Analyses were made on the colloid and on the depleted serums. About one-third of the magnesium in milk was found to be bound to the casein-containing colloid of milk. The concentration of magnesium in this colloid was 0.13%, on the dry basis, corresponding to a mole ratio for calcium to magnesium of 12:1. Comparisons are made to the work and conclusions of others.

M15. Precipitation of calcium caseinate by heat and subsequent reversal. C. A. ZITTLE, Eastern Regional Research Laboratory,[1] Philadelphia, Pa.

Calcium chloride decreases the viscosity of sodium or calcium caseinate solutions, presumably through aggregation since the solutions become white. With calcium less than 0.012 *M* heating to 90° C. for 1 hr. does not change the viscosity, but the solutions become whiter. The whiteness is reversible, at the lower calcium concentrations, when the solutions are cooled. With the calcium above 0.012 *M* the viscosity is increased by heating; on standing at 25° C. the viscosity increase is partly reversed in 1-3 hr. The viscosity increase is due to the formation of a colloidal precipitate of casein; the viscosity decrease is due to the partial re-solution of the precipitate at lower temperatures. The influence of pH and calcium concentration on the amount of precipitate has been studied. Other factors that affect the precipitation and re-solution are time of heating, stirring, and temperature after heating. Cream slows up the re-solution of the colloidal casein precipitate and the accompanying drop in viscosity. It is suggested that formation of a colloidal casein precipitate during sterilization of milk and the slow re-solution during storage accounts for the slow viscosity drop during the storage of evaporated milk.

[1] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, USDA.

M16. A study of the dialysis of skimmilk. G. K. MURTHY and R. McL. WHITNEY. Univ. of Illinois, Urbana.

Interest in the preparation of native protein-free milk systems for the study of milk proteins in their natural environment resulted in a study of the dialysis of an "ocean" of skimmilk against a limited volume of water under conditions such that negligible differences in hydrostatic pressure existed on opposite sides of the membrane at equilibrium. From theoretical considerations and by varying the ratio of water to skimmilk, the following relationship was found between this ratio and the molality of lactose in the dialysate:

$$R = \frac{C\,(\beta m^{\circ}{}_2 - a m_2)\,a m_2}{V\,(a m^{\circ}{}_2 - a m_2)\,[a m_2 - \beta m^{\circ}{}_2 + (A - B)\,\beta m^{\circ}{}_2\,a m_2] - \dfrac{(\beta m^{\circ}{}_2 - a m_2)}{(a m^{\circ}{}_2 - a m_2)}}$$

where R = the ratio of the volume of water to that of skimmilk, A, B, C = constants, $\beta m^{\circ}{}_2$, $a m^{\circ}{}_2$, $a m_2$ = the molality of lactose initially in the milk and initially and finally in the dialysate, respectively, and V = the initial volume of skimmilk.

From this expression and from statistical considerations, the value of the ratio can be selected at which the equilibrium conditions are

not significantly different from zero. The constituents in the equilibrium dialysate of such a system will have the same activities as they possess in the skimmilk and may be considered to be native protein-free milk systems.

M17. Cardboard flavor in skimmilk. D. A. FORSS,[1] E. G. PONT, and W. STARK, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia.

Adding 5 p.p.m. copper as cupric chloride to skimmilk and holding the milk at 2° C. for 3 days produced a flavor described as cardboard, and which resembled milk sunlight flavor in whole milk. The cardboard flavor was proved to be mainly carbonyl in nature, although synthetic work showed that 2-nonenol had a somewhat similar aroma. Investigation of the free compounds and their carbonyl derivatives led to the positive identification of 15 compounds (*n*-hexanal, the C_6-C_{11} 2-enals, and the C_6-C_{11} 2,4-dienals). The most abundant of the unsaturated aldehydes were 2-octenal, 2-nonenal, 2,4-heptadienal, and 2,4-nonadienal and these, together with 2-heptenal, 2,4-hexadienal and 2,4-undecadienal, when added to skimmilk at about $1/10^{7-8}$ produced a flavor very similar to that of "cardboard milk." These compounds were probably derived from traces of unsaturated fatty acids by way of hydroperoxide formation.

[1] Present address: Department of Dairy Science, Pennsylvania State Univ.

M18. Some observations on gamma radiated skimmilk. E. A. DAY, D. A. FORSS, and STUART PATTON, The Pennsylvania State Univ., University Park.

No. 2 cans of raw skimmilk and various fractions therefrom have been studied after radiation by gamma rays at dosage levels of 2×10^6 and 5×10^6 rep. A malty, caramel-like flavor appears to be the prominent off-flavor produced by radiation treatment. However, flavors resembling sunlight, putrid flavor, and the flavor that attends heat-induced browning of milk have been observed, particularly at the higher dosage level. Acetaldehyde as the 2,4-dinitrophenylhydrazone has been isolated and identified as the major volatile carbonyl compound produced by radiation treatment of the milk. Observations on the nitrogen distribution suggest both fragmentation and aggregation of the milk proteins, with increases in the noncasein, proteose-peptone and nonprotein nitrogen fractions and decreases in the albumin-globulin fraction. A significant drop in pH and an increase in titratable acidity have been noted in the skimmilk, in its diffusate and dialysate, and in 5% lactose solutions. The browning reaction is noticeable, especially at the 5×10^6 rep. dosage, and substantial destruction of riboflavin is evidenced by loss of fluorescence in deproteinated filtrates. In addition, activation of sulfhydryl groups is evident, particularly in the dialyzed protein fraction.

M19. Oxidation of vanillin by unheated milk. S. KURAMOTO, ROBERT JENNESS, and S. T. COULTER, Univ. of Minnesota, St. Paul.

Edwards (*J. Assoc. Offic. Agr. Chemists,* **33**: 855. 1950) observed that when unheated milk is incubated with vanillin, a blue color develops upon subsequent treatment with 2,6-dibromoquinonechlorimide (BQC). This reaction appeared to depend on a chemical change in the vanillin induced by an enzyme(s) in milk, but the exact nature of color formation between milk, vanillin, and BQC was not elucidated. The present study was initiated to describe the reaction and to identify the possible nature of the enzyme. Most of the enzymatic activity associated with color development was found to be in the cream portion of milk. Performing the incubation in the absence of oxygen markedly decreased color formation with BQC. Tentative identification of the enzyme as xanthine oxidase was made by comparisons of heat inactivation. Losses of this activity were comparable to losses of xanthine oxidase activity when milk was heated at 70° C. for various times. The absorption maximum of the blue color produced was found to be identical with that of the color formed between vanillic acid and BQC. This is evidence that an initial oxidation of vanillin to vanillic acid and subsequent combination with BQC results in color formation. This procedure is suggested for the estimation of xanthine oxidase activity.

M20. The action of penicillin on lactic acid streptococci in milk. C. K. JOHNS, Canada Dept. of Agriculture, Ottawa, Ont.

Residual penicillin in milk has caused serious trouble in experimental cheese making. Although acid production proceeds normally in the early stages, it subsequently slows down so much as to ruin the cheese. Laboratory studies showed that with 0.15 I.U. penicillin per ml. in the milk, acid production by a strain of *Streptococcus cremoris* proceeded normally for the first 3 hr., then slowed down. The plate count also increased for 1 or 2 hr., later declining sharply. When the milk was initially acidified to 0.35%, acid production was almost entirely inhibited, and the plate count declined from the start. Conversely, when the acidity was neutralized back to the initial value periodically during incubation, bacterial growth and acid development were enhanced. These findings support the hypothesis that the action of penicillin is potentiated by the acidity developed by the lactic streptococci during cheese making.

M21. Host-controlled modification of a bacteriophage without change in poor adsorbability. E. B. COLLINS, Univ. of California, Davis.

Cross-infection with bacteriophages (a method for determining similarities and differences among strains of lactic streptococci) may be misleading if a bacteriophage does not rap-

idly lyse all "susceptible" strains of bacteria. Some bacteriophages were found to be restricted in activity against certain strains of *Streptococcus cremoris*. The activity of some bacteriophages determined in tubes of litmus milk was negative or questionably positive. Most of them became fully active in one growth cycle on a restrictive host.

One restricted bacteriophage did not become fully active in 1 propagation. This restriction was a result of poor adsorbability and weak infectivity of the bacteriophage particles. Adsorbability was not changed during 1 propagation, but infectivity of the bacteriophage particles was changed. Poor adsorption of the progeny by the host prevented a full showing of the change in infectivity that occurred in 1 propagation.

The host was found to contain a very small number of bacterial cells that readily adsorbed particles of the restricted bacteriophage and the progeny. These cells possibly resulted from mutation to "sensitivity"—the mutation involving a change in the bacterial surface that determines adsorption and representing the opposite of the change that occurs in mutation to virus resistance (Luria and Delbruck, 1943). In 1 growth cycle the restricted bacteriophage became fully active on the mutant bacteria.

M22. The germicidal property of milk. R. W. HENNINGSON and F. V. KOSIKOWSKI, Cornell Univ., Ithaca, N. Y.

The germicidal property of milk ("lactenin") has been defined by Jones in terms of its bactericidal effect for Group A streptococci subcultured in this medium. Recently Slade and Slamp have shown that *Streptococcus pyogenes* requires denatured albumin and free sulfhydryl groups for growth in a synthetic medium. Studies on cooked flavor in milk have revealed the appearance of free sulfhydryl groups in milk and whey heated at 75° C. or above for various periods of time. Other investigations show that the germicidal property is eliminated for *S. pyogenes* when milk or whey is heated to 74-80° C. for 20 min. or boiled momentarily.

It has been demonstrated that the germicidal effect of milk for *S. pyogenes*, eliminated by heating, resides in the classical lactalbumin fraction of the milk serum proteins, of which β-lactoglobulin, containing the majority of the sulfhydryl groups in whey, is the major component. No growth of *S. pyogenes* occurred until the milk or whey was heated sufficiently to detect free sulfhydryl groups by the nitroprusside test. On extended heating the free sulfhydryl groups decreased, as did the maximum growth of *S. pyogenes*.

Experiments with *p*-chloromercuribenzoate, cysteine, and native and denatured lactalbumin indicate that a major cause of the germicidal property for *S. pyogenes* is a lack of denatured albumin and/or free sulfhydryl groups in raw milk.

M23. The influence of heating milk on the subsequent response of starter cultures. V. W. GREENE and J. J. JEZESKI, Univ. of Minnesota, St. Paul.

Starter activity, estimated by acid production and population changes, was measured in milks exposed to temperatures ranging from 62° C. for 30 min. to 120° for 60 min. The response of the starters was not uniform with respect to graded severity of exposure but exhibited a cycle of stimulation and inhibition, depending on the intensity of heat treatment. With a constant holding time of 30 min., milk was improved as a culture medium up to exposures of 72°; an inhibitory zone was induced at 80° and 90°; this inhibition was arrested and stimulation again effected at 95° and 120°; further inhibition occurred at exposures more severe than 120° for 30 min.

The effect of heat in the primary stimulation and inhibition cycle could be related to and duplicated by denatured serum protein and cysteine. Though stimulation may be attributed to a variety of factors, inhibition induced by heating below 100° was a unique function of the available cysteine concentration in milk. Further studies on this inhibition phenomenon implicated volatile sulfides that arise from sulfhydryl compounds upon heating.

M24. The response of lactic acid bacteria to stimulants present in pancreas tissue. J. K. MCANELLY and M. L. SPECK, North Carolina State College, Raleigh.

The addition of an aqueous extract of hog pancreas glands to milk resulted in the stimulation of growth and acid production by *Streptococcus lactis* and *Leuconostoc citrovorum*. *Escherichia coli*, *Pseudomonas fragi*, and *Bacillus subtilis* were not stimulated. Two strains of *S. lactis* differed in their response to the extract; the slow-growing strain showed a greater response than did a faster-growing strain. Paper chromatographs of the pancreas extract were prepared and bioautographed on litmus milk agar in order to study qualitatively the differences in response by the 2 cultures. The rapid-growing culture responded to 2 components in the pancreas extract; the slower-growing culture responded to 4 components. In all instances the areas on paper chromatographs that showed the presence of a stimulant also showed the presence of ninhydrin-reactive materials; these materials appeared to be peptide in nature.

M25. Factors affecting terramycin activity in milk.[1] K. M. SHAHANI, The Ohio State Univ., Columbus.

By using a modified disk assay technique, studies were conducted to determine the effect of various factors upon terramycin in milk. Thermal inactivation rates of terramycin in milk, buffer, and water were determined, and

terramycin inactivation was found to follow the first-order reaction in milk or buffer but not in water. The Z values for complete thermal inactivation curves for terramycin, aureomycin, streptomycin, and penicillin revealed terramycin to be the most heat-labile.

Heat inactivation of terramycin in reconstituted milk of different solid concentrations was inversely related to the solids content. Prolonged exposure to nitrogen, air, and oxygen inactivated the antibiotic in milk by 10, 16, and 52%, respectively. The antibiotic seems to be highly labile in the presence of oxygen. Total loss of terramycin (0.3-3.2 γ/ml) was essentially the same in raw and in heated milk (143° and 160° F. for 30 min.) at the end of 5 wk. of storage; i.e., approximately 42-52% was inactivated.

Determination of the effect of raw milk bacteria upon terramycin indicated that the loss of antibiotic potency was directly related to the extent of bacterial metabolism.

[1] Supported by a grant from the U. S. Public Health Service (National Institutes of Health) and by funds from the Ohio Dairy Products Research Fund.

M26. Differences in the phosphorus metabolism of terramycin-sensitive and resistant organisms.[1] K. M. SHAHANI and W. J. HARPER, The Ohio State Univ., Columbus.

Resistance to 100 p.p.m. of terramycin was developed in 4 *S. lactis* cultures. The resistant organisms exhibited normal cellular growth, but their acid-producing ability was retarded. Previous studies on similar *S. lactis* organisms showed differences in the fermentation products of lactose (Angellotti *et al.*, 1955).

Supplementing milk with lactose, lactase, calcium and sodium lactates, glucose-6-phosphate, fructose-6-phosphate, and phosphoglyceric acid had no appreciable effect on the rate of formation or amount of acidity formed by the resistant cultures. Addition of glucose-1-phosphate, or fructose-1,6-diphosphate accelerated the acid development of the resistant organisms.

Tracer techniques showed that inorganic P^{32} was utilized at different rates by the antibiotic-sensitive and resistant *S. lactis* culture. The rate of cellular incorporation of P^{32} was more rapid in the sensitive organisms than in the resistant ones. Radioautograms of autolyzed cells revealed definite variations in the formation of organic phosphate esters by the normal and by the resistant cultures. Three phosphate esters, which were tentatively identified as carbohydrate phosphate esters based on Rf. values, were found in 4-hr.-old cultures of normal organisms but not in the resistant organisms.

[1] Supported by a grant from the U. S. Public Health Service (National Institutes of Health) and by funds from the Ohio Dairy Products Research Fund.

M27. Reduction of cottage cheese biacetyl content by organisms associated with cottage cheese spoilage. C. S. WALES and L. G. HARMON, Michigan Agr. Expt. Sta., East Lansing.

Twenty organisms associated with cottage cheese spoilage were inoculated into the creaming mixture of the cottage cheese and incubated at 40° and 50° F. The biacetyl and acetylmethylcarbinol contents of the cheese were determined colorimetrically, by the method of Prill and Hammer, initially and at 3-day intervals.

Several organisms cause significant reduction in biacetyl and acetylmethylcarbinol. These organisms include species of the genera *Pseudomonas, Escherichia, Micrococcus, Achromobacter, Alcaligenes, Torulopsis, Rhodotorula, Mucor, Penicillium,* and *Geotrichum.* Some of the organisms, of the genera *Micrococcus, Bacillus, Achromobacter, Bacterium,* and *Corynebacterium,* showed no measurable effect upon these flavor components. Reductions were greater at 50° than at 40° F. The amount of reduction by different species and between time intervals was variable.

M28. The effect of various concentrations of sorbic acid at different pH levels on the viability of cottage cheese spoilage organisms. M. D. BONNER and L. G. HARMON, Michigan Agr. Expt. Sta., East Lansing.

Twenty organisms associated with cottage cheese spoilage were tested in trypticase soy broth containing 0.05%, 0.10%, and 0.25% sorbic acid at pH levels of 4.8, 5.0, and 5.2, which represent the range normally encountered in cottage cheese. Growth was determined by measuring turbidity after 48 hr. of incubation at 21° C. Many samples that showed no increase in turbidity exhibited profuse growth when plated on standard agar.

Two species of *Achromobacter* showed reduced viability as pH decreased and sorbic acid content increased. The viability of 2 species of *Escherichia* was reduced with increased sorbic acid but was not influenced by pH within the range studied. Four species of *Pseudomonas* showed considerable variation in tolerance to sorbic acid. *Pseudomonas fluorescens* was not viable at any sorbic acid concentration used and was not viable in the absence of sorbic acid at pH levels below 5.2. *Pseudomonas fragi* showed reduced viability as pH decreased and sorbic acid content increased. The 2 remaining species of *Pseudomonas* studied showed reduced viability as sorbic acid content increased, with pH having no effect on viability. Several other species of microorganisms studied showed considerable variation in tolerance to sorbic acid.

M29. The influence of environment and processing on spoilage organisms in cottage cheese. L. G. HARMON and C. K. SMITH, Michigan Agr. Expt. Sta., East Lansing.

Seventeen vats of cottage cheese were manufactured in 12 commercial plants. Microbio-

logical analyses were performed on samples of all ingredients and of the cheese at various stages of manufacture. When held at 50° F., representative samples from 10 of the vats possessed shelf life ranging from 5 to 10 days, and the shelf life of samples from 7 vats ranged from 13 to 16 days. In the fresh cheese the logarithmic averages of the counts of the coliform, lipolytic, and proteolytic bacteria were 50%, 1,298%, and 1,967% greater in the group of samples with the short shelf life than in the group with the shelf life of 13 to 16 days. The initial organoleptic quality scores averaged only slightly higher in the group with the longer shelf life.

Spoilage organisms were found in samples of "pasteurized" milk and cream, coagulator, starter, water, air, and equipment swabs. Samples of several of the above caused spoilage when inoculated into sterile cheese curd and incubated at 21° C. for 4 days. Several water supplies contained high populations of lipolytic and proteolytic organisms but were free of coliform bacteria.

M30. Withdrawn

M31. The effect of variations in fat and temperature upon the surface tension of various milks. P. D. WATSON, Eastern Utilization Research Branch, USDA, Washington, D. C.

This work on the surface tension of skimmilk, whole milk, and homogenized milk was done in connection with studies on the calibration of lactometers by the National Bureau of Standards. The temperatures of chief interest for this purpose were 60° and 102° F. Some values at the midway point of 81° were determined in order to plot curves.

The measurements were made with the Cenco-du Nouy Precision Tensiometer, which had been carefully calibrated. Determinations of surface tension were made on 72 samples of the various milks at 3 temperatures, and smooth curves were drawn through the points representing average values and the mean deviations.

The results showed that a consistent difference was present between the surface tensions of the 3 classes of milks with an increasing divergence apparent with a rise in temperature, the increase amounting to 50% between skimmilk and whole milk at 102° F. The surface tension of the pasteurized, homogenized milk lay about midway between the other milks. The surface tensions of skimmilk and whole milk at 60° and 102° F. for various fat percentages were plotted, and the results indicated that there was little decrease in surface tension with increased fat percentage above approximately 5% fat.

M32. The use of thermistors for freezing point determinations. W. F. SHIPE, Cornell Univ., Ithaca, N. Y.

A study was made to determine if thermistors could be advantageously used by the dairy industry for cryoscopic measurements. A comparison of methods employing mercury in glass thermometers with an instrument employing thermistors was made to determine their relative merits. A technique for using thermistors for determining the freezing point of milk was developed. This method is easier and much more rapid than the Hartvet technique and gives about the same degree of accuracy. However, the equipment is more complex, and an understanding of the principles of the method requires more scientific training.

The use of thermistors in place of glass thermometers permits the use of a much smaller sample (i.e., 2–3 ml. as compared to 30–35 ml.). This may be a definite advantage when it is difficult to secure large samples. For example, studies of the osmotic pressure of blood and other body fluids will be facilitated.

The application of thermistors to molecular weight determinations will also be discussed.

M33. The influence of composition and treatment of milk upon its freezing point. F. PINKERTON and I. I. PETERS, A. & M. College of Texas, College Station.

Samples of raw and pasteurized milk from individual cows or herds were examined for per cent lactose, conductivity, and freezing point. Lactose was determined by the A.O.A.C. method by using a Rudolph (Routine Model 60) double field polarimeter. Conductivity readings were made with a conductivity bridge (Industrial Instrument Co., Model RC-16 B) and accompanying conductivity cell, and the freezing point was determined with a Hortvet cryoscope.

Whereas similar low freezing points were observed in normal and mastitic milk, the latter was lower in lactose and higher in conductivity. Milk from individual cows ranged in per cent lactose from 5 to 3.7, with freezing points ranging from –0.558° to –0.517° C. In general, the lower lactose milks had the higher conductivity values and the higher freezing points. Storage of raw herd milk for 48 hr. at 0° C. raised the freezing point somewhat and decreased the conductivity. Pasteurization (161° F., 16 sec.) and over-pasteurization (169° F., 16 sec.) of herd milk raised the freezing point and decreased the conductivity with no apparent effect on the lactose present.

M34. The maximum density of milk. C. H. WHITNAH, W. D. RUTZ, and T. M. MEDVED, Kansas State College, Manhattan.

Densities of homogenized herd milk were measured by the hydrostatic method by using an analytical balance and weighted Pyrex glass bulbs having approximate diameters of 28 mm. and volumes of 100 ml. Bath temperatures over the range of –7° to +8° C. were controlled to within 0.001° C. Each sample was placed in a 35 × 250 mm. test tube and was quickly ad-

justed to the desired temperature. The volume and rate of expansion of each bulb were determined by weighing it in water at suitable temperatures and in air of known density.

Irregularities in the density-temperature curve at near 4° C., which were previously reported, were studied with this more precise equipment. They were found to be within the improved experimental error. When milk was supercooled to between –6.25° and –6.75° C., depending on the temperature at which it froze, a maximum density was observed at approximately –5.50° C. The maximum density of milk-water mixtures ranged between –5.50° and 4° C., depending on the percentage of each liquid in the mixture. The maximum density of skimmilk was at about –2.75° C.

M35. Comparison of the Mojonnier, Cenco moisture balance and Karl Fischer titration as methods of determining the total solids in fluid milk. J. B. MICKLE, S. D. MUSGRAVE, R. E. WALTON, F. A. GRAYBILL, M. LOEWENSTEIN, and P. E. JOHNSON, Oklahoma A. & M. College, Stillwater.

A total of 1,448 daily composite samples were collected from 63 Holstein cows over a 20-mo. sampling period. Mean total solids values by the Cenco and Mojonnier methods were statistically different ($P < 0.05$). Duplicates or triplicates were run on 187 Cenco samples and 111 Mojonnier samples. Error variances were 0.172 and 0.033 for the 2 methods, respectively.

In another trial 40 total solids determinations were run from a single milk sample by each of the 3 methods over a 2-day period. Means of 12.80, 13.05, and 12.83 were obtained for the Mojonnier, Cenco, and Fischer methods, respectively. These values were not significantly different ($P < 0.70$). Variances, pooled within days, of 0.0141, 0.0066, and 0.3819 were obtained for the Mojonnier, Cenco, and Fischer methods, respectively. Limits (95%) were set on the differences between means obtained by the 3 methods. On the first day the Cenco values were 0.27 to 0.55% less than the Mojonnier values, whereas values obtained by the Fischer titartion were 0.48 to 0.78% higher than the Mojonnier values. These limits for the second day indicated that the Cenco values were within –0.38 to 0.36% of the Mojonnier values and the Fischer values were within –0.73 to 0.11% of the Mojonnier values.

M36. The protein stability and wettability of milk powder as affected by changes in milk composition. D. CHAKRAVARTI, U. S. ASHWORTH, and H. A. BENDIXEN, Washington State College, Pullman.

Addition of freshly isolated sodium caseinate to concentrated milk before spray drying improved the protein stability but lowered the wettability of the resulting milk powders. The addition of U.S.P. lactose or sucrose improved both protein stability and wettability of the powders. Partial replacement of calcium with sodium by means of an ion exchange resin increased the protein stability but lowered the wettability of the resultant powders. Addition of calcium chloride diminished the protein stability with no appreciable effect on wettability. These results apply to powders stored for 6 mo. at 45° and at 85° F. The chemical composition of each powder was determined. Alcohol stability of the reconstituted powders, which has been shown previously not to be affected by storage, was quantitatively determined at the beginning of storage. Wettability was determined both before and after storage.

Statistical treatment of 95 prepared powders showed a highly significant positive correlation between the lactose content and wettability of the powders. There was also a negative correlation between fat content and wettability. Calcium content and protein stability were inversely correlated, but there was no correlation between wettability and protein stability.

M37. A simplified short-time method for making Cheddar cheese from pasteurized milk. H. E. WALTER, A. M. SADLER, J. P. MALKAMES, and C. D. MITCHELL, Eastern Utilization Research Branch, USDA, Beltsville, Md.

A new method for making Cheddar cheese from pasteurized milk requires only 3 hr. and can be used with conventional equipment, with minor changes in hoops and hooping procedure. The curd is not packed, cheddared, or milled, which greatly reduces hand labor and manufacturing costs.

Two starters are used: the conventional lactic starter, and *Streptococcus durans*, which is salt-tolerant. The milk is ripened at 88° F. for 5 to 60 min. The curd is cut 20 min. after setting. The whey and curd is stirred for 20 min., cooked to 100° F. in 30 min., and stirred for 1 hr. at 100° F. The whey is drained and the curd is stirred until it is quite dry. The dry curd is salted (5 lb. per 1,000 lb. milk). It is hooped in a specially bandaged hoop that has been submerged in either whey or water containing 3% salt at 100°-115° F., pressed for a few minutes with a 20-lb. weight, removed, pressed for 20-30 min. with the weight, and pressed overnight in a conventional press.

The cheese develops a good, mild flavor, has excellent body and texture, is almost free of mechanical openings, and has a uniform volume-to-weight ratio.

M38. The influence of milk-coagulating enzymes of nonanimal origin upon the flavor and constituents of Cheddar cheese. HAROLD WINDLAN and F. V. KOSIKOWSKI, Cornell Univ., Ithaca, N. Y.

A number of rennet-like enzymes of nonanimal origin will coagulate milk, but their ability to make acceptable Cheddar cheese has

never been fully explored. Experimental Cheddar cheeses were made with rennet-like enzymes from mold and bacterial sources and from vegetable sources. Cheese made from calf rennet served as controls. The cheeses were ripened at 50° and 60° F. for periods up to 6 mo. At various intervals samples were analyzed for tyrosine-tyramine concentration, amino acids, small peptides, volatile acidity, and individual free fatty acids.

The use of milk-coagulating enzymes other than rennet has been associated with bitter flavor development in the cheese. In the present work this defect was readily apparent when a rennet-like vegetable enzyme, papain, was used for Cheddar cheese manufacture. Bitterness was so predominant as to make the cheese unfit for consumption.

When rennet-like enzymes from microbial sources were used at optimum concentration smooth curds resulted, and bitter flavors in either curd or cheese were relatively insignificant. Deviation above optimum enzyme concentrations resulted in some bitterness of flavor, which was associated with the appearance of a peptide in the cheese. Differences in the levels of typical Cheddar flavor were observed with cheese in the experimental lots.

M39. Formol titration as a measure of changes in protein during cheese ripening. D. G. VAKALERIS and W. V. PRICE, Univ. of Wisconsin, Madison.

The rate of proteolytic decomposition of ripening blue-mold and Nuworld cheese and a new, soft-ripened cheese was followed by periodic measurements of soluble nitrogen and formol titration in the paracasein-free, sodium citrate-HCl extract of cheese (pH 4.4 ± 0.05) by Mogensen's procedures.

The formol titration of all varieties of cheese increased more rapidly than the soluble nitrogen measured by Kjeldahl analysis. Although the ratios of percentages of soluble nitrogen to formol titration decreased as the cheeses aged, these ratios were remarkably alike at each age within the mold-ripened varieties, despite wide differences in pH and moisture. Other ratios characterized the soft-ripened variety of cheese at each observation period.

The constancy of the ratios characteristic of these varieties at the several stages of ripening suggests the possibility of using formol titration alone to study ripening and to estimate percentages of soluble nitrogen.

M40. Acidic and neutral carbonyl compounds in various cheese varieties.[1] E. W. BASSETT and W. J. HARPER, The Ohio State Univ., Columbus.

Acidic and neutral carbonyl compounds were separated and identified in 82 samples of cheese of 8 major varieties: Swiss, Cheddar, Brick, Blue, Romano, Provolone, Camembert, and Limburger. Sixteen different keto acids and 11 different neutral carbonyl compounds were identified. The most significant finding was that the kind and relative concentration of the acidic carbonyl compounds were characteristic of the cheese variety regardless of its age or flavor intensity. The neutral carbonyl compounds were considered to be degradation products of the acidic components. For each neutral carbonyl compound, a possible acidic precursor was found in the cheese in significant concentration.

Based on the kind of acidic carbonyl compounds found in a cheese, the 8 varieties could be classified into 3 groups. The keto acids characteristic of carbohydrate and citrate metabolism were the major components of the group comprised of Swiss, Cheddar, and Brick cheese. The acids derived from beta-oxidation of fatty acids were characteristic of Blue, Romano, and Provolone cheese. The keto acids derived from deamination of amino acids were predominant in Camembert and Limburger cheeses.

[1] Supported in part by the Research and Marketing Act of 1946, through the Dairy Technology Section, A.R.S., USDA, and by the Ohio Dairy Products Research Fund.

M41. Studies in the ripening of Limburger cheese. S. L. TUCKEY and M. R. SAHASRABUDHE, Univ. of Illinois, Urbana.

Chemical changes that occurred during the ripening of Limberger cheese were measured by chromatographic analyses of the amino acids, organic acids, and carbohydrates. Lactose and glucose persisted until approximately the 4th wk. Galactose usually disappeared between the 2nd and 3rd wk. Amino acids increased in number and quantity during the 12-15 wk. of ripening. By this time the pH had increased from 5.0 to 7.0 or above. The organic acid pattern of Limburger cheese was uniquely influenced by the method of protecting the surface of the cheese, i.e., traditional wrapping or waxing. This in turn regulated atmospheric and moisture conditions on the surface. Lactate increased during the first 4 wk. in wrapped cheese and then decreased so that at the end of ripening almost no lactate was present. However, in waxed cheese from the same lot lactate persisted throughout the ripening period. Moreover, characteristic Limburger flavor developed only in the wrapped cheese. Acetate was present in the fresh curd and persisted at a relatively low level throughout the ripening period. Propionic, butyric, and the fatty acids of C_5 and higher developed during the later stages of ripening (7-8 wk.).

M42. Floating cottage cheese curd made from reconstituted nonfat dry milk solids. A. V. MOORE, A. & M. College of Texas, College Station.

Cottage cheese curd made from reconstituted nonfat dry milk solids floated owing to gas

pockets, most of which were 1/8" to 3/8" in diameter. There was no increase in hole size after dipping, nor did the washed, cooled curd develop an off-flavor after 1 wk. The gassy defect occurred in both short- and long-set batches, under commercial and laboratory conditions. A cutting acidity of 0.55% was regularly attained without delay. Gas-forming organisms were not found in the curd or whey. Prior to reconstituting, the water at 10 commercial sources was boiled. This did not eliminate gas formation in the curd. Water at 3 plants ranged in pH from 7.5 to 8.4 and varied widely in mineral content. Gassy curd was eliminated at these plants when the water was adjusted to pH 6.8 with sulfuric acid. The curd did not cook as well when the water was adjusted with citric or lactic acid. Seasonal analyses of natural water at 20 commercial sources indicated that versenate hardness, bicarbonate, and chloride were the most variable factors. Base exchange water softening eliminated gassy curd but also produced an undesirable softness.

M43. The use of synthetic β-carotene for coloring butter. R. R. RIEL and C. K. JOHNS, Dairy Technology Unit, Canada Dept. of Agriculture, Ottawa.

Synthetic β-carotene was compared to coal-tar dye as coloring agent for butter. The butter was made from split vats of sweet and sour cream produced during the winter season. Flavor, hydrolytic and oxidative changes, color intensity, and vitamin A potency were determined initially and after 2 wk. at 50° F., 6 mo. at −5° F., and 6 mo. at −5° F. followed by 2 wk. at 50° F.

Neither official graders nor a taste panel of 20 members could detect any flavor difference. The acid degree and the peroxide and aldehyde values were the same for both types of butter. The color intensity of the butter, judged by Hansen's chart, Richardson's method, or spectrophotometrically, did not vary during storage. The vitamin A potency was increased by an average 3,200 I.U. per lb. as a result of coloring the butter with β-carotene, and was not affected by storage.

M44. The influence of various packaging materials on the keeping quality of ice cream and on rates of temperature change during hardening and subsequent handling. V. S. PACKARD, JR., E. L. THOMAS, and W. B. COMBS, Univ. of Minnesota, St. Paul.

In comparative studies of conventional packaging materials, aluminum foil laminates and overwraps afforded superior protection to ice cream stored for 1 mo. in self-service ice cream cabinets. Shrinkage and objectionable coarseness of texture, which frequently occurred in fiber and plastic containers, were absent in foil-covered containers. The most pronounced differences were noted in containers stored at the top level of open-top and sliding-glass-top cabinets. Temperature measurements at this level revealed that ice cream in foil-wrapped containers was maintained at temperatures averaging 9° F. lower than that in conventional containers.

Foil coverings tended to retard the rate of hardening in comparison with fiber containers, but the differences were not significant. Rates of temperature rise were slightly less in foil-covered containers than in fiber containers when exposed to room temperatures. The insulating ability of various types of insulated bags and sleeves for ice cream containers was also investigated. The effectiveness of aluminum foil as a packaging material for ice cream is explainable in terms of its high thermal conductivity and its being a good reflector, but poor radiator, of radiant heat.

M45. A process for manufacturing a high nitrogen feed supplement from whey. D. R. ARNOTT, S. PATTON, and E. M. KESLER, The Pennsylvania State Univ., University Park.

Experiments have been conducted to determine the possibility of treating liquid Cheddar cheese whey with ammonia in order to produce a feed product with increased nitrogen content. To enhance ammonia-binding capacity the whey was "ripened," with *Lactobacillus bulgaricus* and a yeast of the genus *Mycoderm*, to a titratable acidity of 1.30-1.70%. Anhydrous ammonia was then introduced with a Penberthy injector until the titratable acidity was reduced to approximately 0.80%. Acid production was then allowed to proceed, followed by neutralization. This pattern was repeated until the lactose content of the whey was reduced to approximately 1%. As a result of this treatment the nitrogen content of whey was increased 3.5-6.0 times over original levels. At this point the whey was condensed to a total solids level of approximately 30%.

Feeding trials were conducted with 2 lactating Holstein cows in order to determine the palatability and toxicity of the ammoniated whey product. The condensed whey was fed at levels of 10, 20, and 30% by weight of the concentrate being fed. The animals accepted the feed with no apparent ill effects on physical condition or milk yield.

M46. Evaluation of the cleanability of CIP Automatic valves.[1] D. A. SEIBERLING and W. J. HARPER, The Ohio State Univ., Columbus.

The introduction of automatic cleaning processes to the dairy industry has revealed the desirability and the feasibility of remote-operated valves that can be reliably cleaned in place. The development of such valves is also a prerequisite to further automation. In order to determine the cleanability of three experimental remote-actuated valves, radioisotope tracer techniques were employed in addition to standard methods.

Radioactive P^{32} had been used by other investigators to evaluate the cleaning of glass pipelines. In this study it was found that P^{32} was not satisfactory since it reacted irreversibly with the stainless steel. Ca^{45} was found to accumulate in milk films and milkstone deposits, which could be completely removed by adequate cleaning. Relative cleanliness was indicated by the absence of isotope-laden films as indicated by use of a thin-window Geiger counter and by radioautography.

Comparison to bacteriological examination showed that the isotope procedure was more sensitive in detecting soil. Traces of Ca^{45} were detected on surfaces shown to be bacteriologically clean.

[1] Supported in part by Ohio Dairy Products Research Fund.

PRODUCTION SECTION

P1. The influence of oxytocin and epinephrine upon uterine motility and metabolism. M. A. Inchiosa and N. L. VanDemark, Univ. of Illinois, Urbana.

The rapidity with which oxytocin and epinephrine act upon the uterus in vivo suggests that these hormones have a profound influence in producing a biochemical reaction. In an attempt to obtain information on the metabolic role of these hormones in uterine muscle, studies have been conducted on the muscle lever and tissue homogenates and extracts.

Aerobic and anaerobic studies of uterine motility and metabolism have suggested an influence of oxidation-reduction potential upon contractility and phosphorus metabolism. The relative effect of oxytocin in increasing uterine contractions was much greater under anaerobic conditions. Glutathione and sodium thioglycollate, in strong reducing concentrations, were found to inhibit uterine motility in muscle lever experiments. This inhibition was reversed by the addition of oxytocin. Another reagent, *p*-chloromercuribenzoic acid, which is known to inhibit certain muscle enzymes, was found to decrease the phosphorus metabolism of uterine extracts. Epinephrine, under controlled reducing concentrations, was found to increase the phosphorus metabolism of uterine muscle extracts. Preliminary evidence suggests that this action is a stimulation of myosin and actomyosin adenosine triphosphatase activities.

P2. The induction of estrus in cows by electrical stimulation. R. L. Hays and C. H. Carlevaro, Univ. of Illinois, Urbana.

It was noted by casual observation that cows often come in heat after manual examination of the reproductive organs. It was decided to test the idea that stimulation of the reproductive organs would produce estrus by using an apparatus designed for the electro-ejaculation of bulls. An attempt was made to place the electrodes, which were parallel rings on a rubber hose, in the vicinity of the cervix, uterus, and ovaries after insertion into the rectum. Estrus was induced in 6 cows which had not shown signs of heat for 42-166 days. Estrus was induced also in cows which had not been in heat for 50-60 days after calving. Although only small numbers of animals have been involved to date, all of the cows stimulated have come in heat 1-7 days after stimulation. Palpation and slaughter have shown that in each case a corpus luteum was formed and presumably ovulation had occurred. Experiments are in progress to determine the fertility of cows bred during the induced heat and to study the effects of electrical stimulation in sheep and goats.

P3. Luminal contents of estrous and pseudopregnant rabbit uteri as bacterial culture media. H. W. Hawk, J. Simon, S. H. McNutt, and L. E. Casida, Univ. of Wisconsin, Madison.

The uteri of luteal-phase cows and rabbits show much greater numbers of bacteria still alive at a standard time after inoculation than do the uteri of follicular-phase animals. This differential might be due to differences in the uterine defensive mechanisms; however, leucocytes from both types of inoculated uteri often contain large numbers of ingested bacteria. Another possibility might be that uterine secretions differ as culture media.

Both uterine horns of 8 estrous and 8 pseudopregnant rabbits were ligated at each end and 1 horn was excised. Each horn was inoculated with approximately equal numbers of *Escherichia coli* and the excised horn was left in the peritoneal cavity with the intact horn. The difference in total bacteria recovered from the 2 kinds of intact horns at the end of 4 hr., mean log of 6.54 for the estrous and 8.30 for the pseudopregnant, was greater ($P < 0.01$) than that from the excised, 8.22 for the estrous and 8.37 for the pseudopregnant; this fails to support the hypothesis of a difference in properties as a culture medium. A difference in the defensive mechanisms other than complete inability of leucocytes to ingest bacteria remains a possible explanation.

P4. Progesterone and B vitamins as factors in maintaining pregnancy in the underfed rat. K. A. Kendall and R. L. Hays, Univ. of Illinois, Urbana.

On the day of mating, female rats were given distilled water and 1 of 4 test diets, namely: (1) complete, (2) complete less added minerals, (3) sucrose plus fat, and (4) sucrose. Animals were sacrificed on the 20th day after mating. Average litter size per mating for diets 1, 2, 3, and 4 was 10.1, 9.4, 5.0, and 1.1 young, respectively. Early termination of pregnancy resulted with all diets when progesterone (5.0 mg.) and estrone (5.0 γ) were injected daily.

Pregnancy was maintained on diets 1 and 2 with injections of progesterone (5.0 mg.) and estrone (0.5 γ); average litter size per mating was 7.25 and 5.0, respectively. Average litter size per mating on diets 3 and 4 with progesterone (5.0 mg.) was 5.7 and 7.12, respectively. Average litter size per mating on diet 3, with B vitamins added, was 7.6, and on diet 4 with progesterone (5.0 mg.) and estrone (0.5 γ) the number of young per mating was 7.12 with B vitamins added and 7.13 without the vitamins. The percentages of dry matter, total nitrogen, and ash in the fetuses were constant regardless of diet, although fetus weights varied as much as 200% between diets.

P5. Split-ejaculate fertility comparison between unfrozen and frozen bull semen. J. A. WILLIAMS, Michigan State Univ., and R. W. GREEN, Mich. Artificial Breeders Coop., East Lansing.

Conception rates on 53 bulls, representing 7 breeds, were obtained during the fall of 1955. Fifteen inseminators cooperated in the study. Unfrozen and frozen semen from the same ejaculate were used during the first and last half of the month, respectively. The extender used was heated homogenized milk. Penicillin and streptomycin were added. The unfrozen semen was shipped 5 days per wk. The semen for freezing was extended to give a minimum of 10 million live sperm after thawing. Glycerination was 7% by volume and equilibration was 18 hr. Storage was at –76° C. in polyethylene plastic ampules in 1.1-ml. amounts. Approximately 2 wk. elapsed between usage of the unfrozen and frozen portions. The frozen semen was thawed in ice water at 5° C. prior to insemination. The 60-90 day nonreturns on 979 unfrozen and 1,207 frozen samples used for first services were 73.9 and 65.3%, respectively. The difference was highly significant. Technicians' results varied from 61 to 85% with unfrozen semen, and from 52 to 79% with frozen semen. Considerable variation among bulls was evident. The frozen semen gave conception results comparable to those with unfrozen semen used over the week ends.

P6. The effect of dilution temperatures and cooling rates upon the survival of frozen and unfrozen sperm. E. W. SWANSON and R. H. WHITE, Univ. of Tennessee, Knoxville.

Ten samples of bull semen were diluted in each of 3 diluents at 60°, 70°, 80°, 90°, and 100° F. within 5 min. after collection. After being held at these temperatures for about 15 min., the samples were transferred in insulated tubes to a 40° F. refrigerator. Motility ratings made initially and for 10 days in storage averaged 44.9, 46.9, 49.0, 51.1, and 46.8% for the respective temperatures. Differences due to temperature were highly significant. Eighteen samples of semen to be frozen were diluted initially at 60°, 70°, 80°, 90°, and 100° F. Revival rates after freezing were slightly higher from 90° F. diluents than from 80° or 70° F. diluents and markedly above those from 60° or 100° F. dilutions. Cylinders of diluted semen to be frozen were cooled from 90° to 40° F. in a 1,500-ml. water bath, in insulated cylinders, and in air unprotected. Times required to reach 40° F. were 6, 3.5, and 1.7 hr., respectively. Average revival rates of 4 samples 7 days after freezing to –110° F. were 59.8% from the water bath and 52.7% from the insulated tube cooling. These results indicate that the effects of dilution temperature and cooling rate upon the resistance of bull sperm are comparable in 40° F. storage and in freezing.

P7. The effect of collection interval upon some characteristics of bovine spermatozoa and their survival through freezing. F. I. ELLIOTT, E. J. ELLIOTT, J. I. OHMS, and E. L. WILLETT, American Fdn. for the Study of Genetics, Madison, Wis.

Each of 8 mature bulls of the Brown Swiss, Guernsey, and Jersey breeds was "exhausted" of sperm 8 times by means of 5-16 successive ejaculations at 10-min. intervals. A bull was considered to be exhausted when he gave 2 successive ejaculates each containing fewer than 1 billion sperm. After an interval of 1, 3, 5, or 7 days following each exhaustion a single ejaculate was collected. Each single ejaculate collection was followed by a 7-day rest and another exhaustion. Thus, the design of the experiment was that of a randomized complete block with each bull being collected twice after each of the post-exhaustion intervals. The latter caused highly significant differences in several semen characteristics. Significantly greater volumes of semen and numbers of sperm were produced after the 7-day interval. Post-freeze motility was highest after the 5-day interval. There were no significant trends in number of cytoplasmic droplets on, or light reflecting properties of, sperm with successive ejaculates or with increase in collection interval.

P8. The daily rate of sperm production in dairy bulls. L. J. BOYD, and N. L. VANDEMARK, Univ. of Illinois, Urbana.

Numerous reports indicate that the restoration of sperm numbers in the bull is rapid after exhaustive semen collections. No reports specify the daily rate of sperm production. Preliminary trials with 2 bulls showed that the total sperm ejaculated remained fairly constant with intervals of 7 days between partial exhaustions (10 consecutive ejaculates within 90 min.). Sperm numbers were not increased by extending the interval to 30 days.

A twice replicated 3 × 3 Latin square design involving 6 bulls was used in an attempt to establish the daily rate of sperm production. Each bull was subjected to partial exhaustion at intervals of 1, 4, and 7 days after an initial

partial exhaustion. Thus, 40 ejaculates were collected from each bull in 13 days.

The average number of sperm obtained in the initial partial exhaustion was 19.5×10^9. An average of 7.8, 13.5, and 19.5×10^9 sperm was produced after intervals of 1, 4, and 7 days, respectively. Regardles of the interval between partial exhaustions, approximately 6 billion sperm were obtained plus 2 billion for each additional day. Again, complete restoration of the severely depleted sperm supply was accomplished in 7 days.

P9. Bicarbonate buffer, glycine, and versene in diluters for bull semen. E. L. WILLETT and J. I. OHMS, American Fdn. for the Study of Genetics, Madison, Wis.

Livability of bull spermatozoa has been studied in 4 experiments in which bicarbonate buffer, glycine, and versene were constituents of diluters. Each experiment consisted of 12 or 14 ejaculates split to compare 6 to 9 different treatments. In all treatments dihydrostreptomycin sulfate was included at a level of 500 γ per ml. After storage at 38° C. for 2, 4, and 10 days, motility observations were made for each sample by 2 people independently, and the estimations were averaged. When diluted 1:9, spermatozoan viability in yolk-bicarbonate-glucose (YBG) was not significantly different from viability in yolk-citrate (YC), but when diluted to 15 million spermatozoa per ml. viability was inferior in YBG. Initially, pH of YBG is 7.3-7.5 but when diluted 1:9, pH is reduced during storage to 7.0 and lower by metabolites produced by spermatozoa. When diluted to 15 million spermatozoa per ml., pH is lowered only slightly. Adjusting pH of YBG to 6.7 or 6.8 before adding spermatozoa improved viability slightly. Addition of glycine or versene to yolk-citrate did not improve viability of spermatozoa when compared with viability in yolk-citrate alone.

P10. Mineral levels of bovine semen and seminal plasma. R. G. CRAGLE and J. H. MUNTZ, Univ. of Illinois, Urbana.

Little is known of the importance of mineral levels in bovine semen or the relationship of the level of these elements to spermatozoan activity. To establish a basis upon which further studies could be conducted, mineral levels were determined in whole semen and seminal plasma, including levels of boron, phosphorus, magnesium, copper, and iron by means of the Emission Spectrograph, and levels of sodium, potassium, and calcium by the use of the Coleman 21 Flame Photometer.

Composite samples of several consecutive ejaculates, collected over a period of 40 min. or less, were used to obtain sufficient volume for the complete analysis from each bull on a given composite sample. Preliminary average values of 8 composite samples in mg. per 100 ml. for whole semen and seminal plasma, respectively, were: boron 1.74, 1.73; phosphorus 79, 40; magnesium 9.8, 9.8; copper 0.106, 0.052; iron < 0.1, < 0.01; sodium 240, 256; potassium 149, 145; and calcium 21, 22. Phosphorus, copper, and iron values indicated that these elements were more concentrated in the spermatozoa than in the seminal plasma.

P11. Motility of sperm in milk diluent with and without seminal plasma. J. L. ALBRIGHT, M. H. EHLERS, and R. E. ERB, State College of Washington, Pullman.

Sperm are reportedly not capable of utilizing lactose as a source of energy. A study was conducted to determine whether sperm in the absence of seminal plasma would retain their motility in heated homogenized milk. Sperm were removed from bull semen samples by centrifugation and the plasma was replaced with the same amount of sodium citrate buffer. In 1 experiment, only the supernatant plasma was removed; in another, sperm were twice washed with citrate. Restoration to volume is referred to as semen-equivalent. Semen-equivalent was diluted with 0 (A), 0.5 (B), 1 (C), 2 (D), and 4 (E) parts milk, and with 1 part plasma (F). The last dilution served to approximately restore the original sperm-plasma relationship. All dilutions were then brought to the same total volume with citrate buffer to represent a 1:4 semen-equivalent dilution.

Samples were placed in a 37° C. water bath and were evaluated for motility, initially and hourly for 3 hr. Samples without milk or restored plasma showed very low progressive motility after 1 hr. During 3 hr., motility of the nonwashed sperm group decreased 85% (A), 68% (B), 57% (C), 49% (D), 39% (E), and 56% (F). With the twice-washed sperm, motility decreased 100% (A), 81% (B), 56% (C), 61% (D), 55% (E), and 54% (F). In other comparisons whole semen maintained excellent motility at 37° C. for 3 hr. in the varying milk diluters. These results indicate that sperm are capable of utilizing certain constituent(s) in milk as a source of exogenous energy.

P12. Uptake of phosphorus^{32} from egg yolk phospholipids by bovine spermatozoa. M. D. CRAWFORD, R. J. FLIPSE, and J. O. ALMQUIST, Pennsylvania Agr. Expt. Sta., University Park.

Labeled phospholipids were extracted from the eggs of a hen after intra-peritoneal administration of sodium phosphate-P^{32}. Immediately before each incubation trial, an emulsion of the phospholipids in Ringer-phosphate was prepared. Whole semen samples or thrice-washed spermatozoa were suspended in this medium to a concentration of 0.5×10^9 cells per ml. and were then incubated aerobically at 37° C.

Washed spermatozoa consistently took up at least twice as much P^{32} as did whole semen or spermatozoa resuspended in seminal plasma after washing. Addition of fructose (final con-

centration of 0.03 *M*) to washed spermatozoa tended to increase P^{32} uptake and thus the lowered counts in whole semen cannot be attributed to its fructose content. When metabolic inhibitors were added to reduce the elimination of P^{32} by spermatozoa, cellular P^{32} counts were increased 2 or 3 times in washed sperm preparations but were essentially unchanged in whole semen. Addition of fructose to metabolically-inhibited washed spermatozoa did not effect P^{32} uptake.

P13. The metabolism of bull semen at low temperatures in saline- and phosphate-containing diluents. A. W. BLACKSHAW and G. W. SALISBURY, Univ. of Illinois, Urbana.

The aerobic metabolism of bull semen was studied at temperatures of 37°, 21°, and 5° C. The oxygen uptake of semen diluted 1:4 with isotonic sodium chloride or isosmotic sodium phosphates adjusted to pH 7 was greatly reduced at 21° and 5° C. At 37° C. the oxygen uptake in the saline was much greater than in the phosphate-containing diluent, but at 21° and 5° C. the rates in both diluents did not differ significantly.

Fructose utilization in saline at 37° C. was only about 60% of that in the phosphate, whereas lactic acid accumulation was 50%. However, at 21° and 5° C. there were no significant differences between the diluents for these two characters. At all temperatures the viability of the cells was depressed by the phosphate diluent.

Temperature shock reduced the oxygen uptake of bull semen incubated at 21° and 37° C., but the rate of uptake did not differ between diluents. The accumulation of lactic acid and the disappearance of fructose was reduced in cold-shocked semen incubated at 37° C., but the effect was greater for fructose utilization than for lactic acid production.

P14. Pathways of glucose catabolism in bovine spermatozoa. R. J. FLIPSE, Pennsylvania Agr. Expt. Sta., University Park.

In attempts to gain information regarding the possible occurrence of a nonglycolytic pathway of glucose utilization in bovine spermatozoa, the yields of carbon[14] labeled carbon dioxide from glucose-1-C^{14} and uniformly labeled glucose (glucose-U-C^{14}) have been compared. The procedure is based on the production of CO_2 preferentially from carbon 1 of glucose in the known alternate pathways of glucose catabolism in mammalian tissue. Twice-washed spermatozoa were suspended in Ringer-phosphate to a final concentration of 0.5×10^9 cells per ml. within 2 to 3 hours after collection. Incubation was at 37° C. with air or nitrogen as the gas phase. In each trial, one aliquot was incubated with glucose-U-C^{14} and a second aliquot with glucose-1-C^{14}. Carbon dioxide was collected with filter paper and potassium hydroxide in the center well. After incubation, the collected CO_2 was converted to $BaCO_3$ and the yield of $C^{14}O_2$ (as per cent of labeled substrate) was determined. In 4 of a series of 8 experiments involving single ejaculates the ratio

$$\frac{\%\ C^{14}O_2 \text{ from glucose-U-}C^{14}}{\%\ C^{14}O_2 \text{ from glucose-1-}C^{14}}$$

approximated unity, whereas in the other 4 it was considerably less than unity (0.36-0.85). Thus, in some ejaculates a considerable part of the CO_2 derived from glucose evidently arose by nonglycolytic pathways.

P15. Viability of spermatozoa in bovine genital tract fluids. DURWARD OLDS, Univ. of Illinois, Urbana.

Cervico-vaginal mucus, uterine fluid, oviduct fluid, and follicular fluid were collected from 12 cows slaughtered at known stages of the estrous cycle. Similar fluids were collected and partially pooled from 9 cows at uncertain stages of the cycle. Viability of spermatozoa in these fluids was tested by drawing a small quantity of test fluid into a capillary tube, following this with a small bubble of air and then a short column of semen, after which both ends of the tube were plugged with vaseline. The filled tubes were incubated at 37° C. and examined periodically for spermatozoan motility. In general, it appeared that spermatozoa could live about 10 hr. in mucus, 6 hr. in uterine fluid, 12 hr. in oviduct fluid, and 20 hr. in follicular fluid. Viability in follicular and oviduct fluids was not appreciably affected by stage of cycle. However, in mucus and uterine fluid, viability appeared to be somewhat better during or near estrus. Agglutination of spermatozoa was observed in all of the fluids and at various stages of the cycle. It was perhaps more extensive and more regularly produced by follicular fluid. Follicular fluid appears to stimulate both motility and oxygen uptake of spermatozoa.

P16. The metabolic activity of semen collected in different seasons of the year. N. T. NAKABAYASHI and G. W. SALISBURY, Univ. of Illinois, Urbana.

Earlier studies indicated that an association existed between the seasons of the year and metabolic behavior of semen. Thus, in this study, the metabolic activity of sperm cells from semen collected during a winter and a summer season was compared. A part of each semen sample was washed and resuspended in its own plasma and also in plasma produced by the same bull but in the preceding season. Ejaculates from 9 bulls representing the Holstein-Friesian, Guernsey, and Ayrshire breeds were obtained and replicated each season.

The oxygen uptake, fructose utilization, or lactic acid formation of washed winter cells were not significantly altered ($P > 0.05$) by the plasma of different seasons. In like manner, the metabolic response of washed summer

cells was not significantly affected ($P > 0.05$) by the plasma of different seasons. However, washed winter cells utilized 62% more fructose and formed 69% more lactic acid than summer cells. These differences were highly significant ($P < 0.01$). The oxygen consumption of summer cells was slightly more than that of winter cells but the difference was not statistically significant.

It appeared that seminal plasma had little influence on fructolysis in ejaculated semen. The seasonal differences observed seem to be primarily inherent in the sperm cells.

P17. Evaluation of the thyroid activity of dairy animals.[1] G. W. PIPES, Univ. of Missouri, Columbia.

In order to study the relationship between thyroid function, milk production, and other physiological processes, methods for evaluation of thyroid activity have been investigated. The application to ruminant animals of medical techniques for in vivo determination of thyroidal I^{131} has allowed precise measurements.

Three criteria of thyroid function have been utilized: the rate of release of thyroidal I^{131}, the rate of release of thyroidal I^{131} when goitrogens were administered to prevent the reutilization of I^{131} from metabolized thyroid hormone, and the estimation of the daily thyroid secretion rate by replacement therapy. The last method depends upon the administration of graded doses of L-thyroxine to inhibit the secretion of thyrotrophic hormone, which in turn inhibits the secretion of thyroid hormone, as shown by reduced rates of loss of thyroidal I^{131}. Thiouracil or methyl thiouracil has been administered at the rate of 1 g. per 100 lb. body wt. to increase the sensitivity of the method.

Preliminary investigations made by these methods have shown a significant degree of correlation between the thyroid activity of dairy animals and peak milk production during subsequent lactation.

[1] Contributions from the Department of Dairy Husbandry, Mo. Agr. Expt. Sta., Journal Series No. 1613. Approved by the Director. These investigations were supported in part by grants of the U. S. Atomic Energy Commission.

P18. The role of the pituitary gland in mammary gland growth. R. P. REECE, New Jersey Agr. Expt. Sta., New Brunswick.

In the past hypophysectomized animals have been widely used in attempting to identify the anterior pituitary factor required for mammary growth. For extensive testing it would be desirable if an assay procedure could be developed that was not based on hypophysectomized animals. Such a procedure will be described and evidence will be presented indicating that the lactogenic hormone may be the anterior pituitary factor required for mammary growth.

P19. Stimulation of udder growth and lactation in infertile dairy heifers.[1] H. L. RUPPERT, JR., H. YAMAMOTO, and C. W. TURNER, Univ. of Missouri, Columbia.

A preliminary report of this work was presented last year. Nine heifers have now been treated with a combination of estrogen and progesterone to induce growth of the mammary gland, followed by the injection of increased amounts of estrogen to initiate lactation. These included 7 sterile heifers, 1 freemartin, and 1 heifer of a set of normal identical twins. The other twin heifer was bred and calved normally.

Eight heifers that received this hormone treatment were induced to lactate to a degree comparable to that of related animals. The yield of milk at the period of maximum production was used as an index of the extent of gland growth stimulated. The only failure to induce lactation occurred in a freemartin Guernsey heifer. Additional freemartins will be used as they become available.

When the peak of milk production began to decline, these heifers were fed 10 mg. of diethylstilbestrol daily for 4 wk. In all animals, the declining trend in milk production was arrested, and in several animals production began to increase again. This treatment was followed by the daily injection of thyroxine for 4-8 wk., which further increased milk production.

[1] Contribution from the Mo. Agr. Expt. Sta., Journal Series No. 1611. Approved by the Director.

P20. Some effects of feeding diethylstilbestrol to dairy cattle. C. B. BROWNING, G. B. MARION, F. C. FOUNTAINE, and H. T. GIER, Kansas State College, Manhattan.

Twenty-two dairy cows were fed diethylstilbestrol daily at levels varying from 5 to 20 mg/1,000 lb. body wt. Reproduction apparently was not affected. Eight cows fed diethylstilbestrol for an average of 40 days averaged 1.5 services per conception. One of these had been previously bred twice, and another, 3 times. Five cows, pregnant 3-5 mo., were fed diethylstilbestrol 78-190 days and calved normally. Another cow, pregnant 10 days, was fed diethylstilbestrol for 110 days, and has a living fetus. Two others have not conceived, one from 2 services and the other from 3, after 3 mo. of diethylstilbestrol feeding. Among the 22 cows fed diethylstilbestrol, none showed abnormal physical changes or estrous cycle irregularities, except that a few cows had slight swelling and hypervascularization of the vulva within the first 20 days of diethylstilbestrol intake. Although the effects of diethylstilbestrol feeding on milk production are inconclusive, data with 5 pairs of identical twins indicate a slight increase in production. Adrenal

and pituitary glands obtained from 6 of the cows fed diethylstilbestrol for 3 mo. showed no gross or microscopic evidence of abnormality.

P21. Effect of feeding stilbestrol to lactating dairy cows. J. F. SYKES, T. R. WRENN, E. A. KANE, and W. C. JACOBSON, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Stilbestrol was fed daily at the rate of 10 mg. per day to 6 lactating cows for a 60-day period. Four cows were at mid-lactation at the start of the trial and 2 were near the end of lactation. Milk production ranged from 20.0 to 35.0 lb. per day at the start of the trial. Three of the cows were fed a ration of alfalfa hay and corn silage and 3 were fed grass-legume silage. All cows were fed a 15% protein concentrate according to milk production. No effect on milk production as a result of stilbestrol feeding was observed. The normal decline in milk production proceeded without change during the experimental period. There was likewise no change in lactation when stilbestrol feeding was discontinued.

The effect of feeding stilbestrol on the digestibility of the alfalfa hay–corn silage ration was determined. Digestibility of the dry matter of the ration tended to be improved, but the difference was not statistically significant. Crude fiber digestibility increased when stilbestrol was fed, and protein digestibility decreased.

This experiment indicates that, in spite of the marked effects produced in beef cattle by feeding stilbestrol at the rate of 10 mg. per day, this procedure has little or no application for increasing milk production of dairy cows.

P22. Estrogen content of milk of open and pregnant cows and effect of synthetic estrogen feeding.[1] C. W. TURNER, Univ. of Missouri, Columbia.

It was thought of interest to determine the amount of estrogen that might be present in cow's milk. This was done by using young ovariectomized mice, whose uteri are very sensitive to estrogen even by oral administration. Milk samples were obtained from open and pregnant cows, dried under infrared lamps, and assayed by feeding to mice. For comparison, milk from cows fed various levels of synthetic estrogens was assayed to determine the possible passage of these estrogens into milk.

The average uterine wt. of ovariectomized mice fed estrogen-free food varies between 8 and 12 mg. Feeding of commercial milk resulted in uterine weights averaging between 17 and 18 mg., indicating the presence of small amounts of estrogen. The milk of nonpregnant cows showed the presence of little or no estrogen. The average uterine weight of mice fed milk from pregnant cows varied from 43.53 mg. for a cow pregnant 222 days and producing 7.2 lb. of milk to a low of 11.71 mg. for a cow pregnant 178 days and yielding 21.9 lb. per day. Milk from about 50% of the cows pregnant 150 days or more resulted in uterine weights averaging 20 mg. or more.

The milk of nonpregnant cows fed 10 mg. of diethylstilbesterol per day indicated the presence of little or no estrogen in the milk.

[1] Contribution from the Mo. Agr. Expt. Sta. Journal Series No. 1610. Approved by the Director.

P23. The mode of formation of milk fat.[1] S. LAKSHMANAN and J. C. SHAW, Univ. of Maryland, College Park.

Tracer experiments show that the synthesis of short-chain volatile fatty acids (VFA) of milk fat represents the bulk of ruminant mammary lipogenesis (ML). The rest of the milk fat is derived from blood lipids. The production of VFA over a given interval is a measure of ML, and the VFA content of milk fat is a measure of the ratio of ML to blood fat uptake. The total output of fat is the sum of these sources of fat. It can be shown that ML is dependent on the availability of acetate and β-hydroxybutyrate to the gland and that the direct reduction of β-hydroxybutyrate is less sensitive to experimental alteration than the condensation of C_2 units. The variations in milk fat output and fat composition induced by fasting, cod liver oil feeding, low roughage feeding, injections of GH and ACTH, and dietary fat are explicable on this basis.

Data on early effects of fasting, studied by using short interval milking as a physiological technique, will be presented showing that *(a)* there is no relationship between VFA content and the rate of milk fat secretion, *(b)* fasting inhibits ML, *(c)* adipokinesis facilitates fat uptake by the gland and compensates for the depression in ML, and *(d)* the existing theories, except the present one, cannot explain the data.

[1] Work supported by U.S.A.E.C.

P24. The preparation of carbon^{14} labeled uridine diphosphate glucose for a study of lactose metabolism. S. K. MURTHY and R. G. HANSEN, Univ. of Illinois, Urbana.

Uridine diphosphate glucose (UDPG) has been obtained enzymatically in good yields from carbon^{14} glucose-1-phosphate and uridine triphosphate (UTP). The enzyme was isolated from *S. fragilis,* and the carbon^{14} glucose-1-phosphate was prepared from carbon^{14} starch by equilibration with phosphorylase. The starch was isolated from plants that had respired in an atmosphere of $C^{14}O_2$. The level of radioactivity of the UDPG is only limited by the activity of the glucose-1-phosphate since no dilution of the hexose takes place during synthesis by this procedure. The activity of one

such preparation was 18,400 counts per min. per γM of hexose. The purity of the product has been verified by ion exchange chromatography and by ionophoresis. Yields vary from 60 to 75% of the purified UDPG based upon added UTP. Twenty γM of substrate have been employed in this procedure although the preparation is readily adaptable to larger scale operations.

P25. Appearance of the specific blood serum proteins in the lacteal secretions near parturition. B. L. LARSON, G. D. ROLLERI, and K. A. KENDALL, Univ. of Illinois, Urbana.

The specific proteins present in the lacteal secretions near parturition were studied on cows milked daily through the normal 2-mo. dry period. The lacteal fluid from dry cows also was examined at weekly intervals. Simultaneously with the appearance of large amounts of the immune euglobulin and pseudoglobulin components in the lacteal secretions, the level of the γ_1- and the β_2-globulins began to decrease in the blood serum. Since the properties of these proteins are similar, this suggests that the euglobulin and pseudoglobulin constituents of colostrum (and milk) are identical with the γ_1- and β_2-globulins of blood, respectively.

A more gradual decrease in the level of the α-globulins also occurred in the blood before parturition; proteins with the properties of the α-globulins were found in the lacteal secretions. Trace amounts of proteins with the properties of the blood γ_2- and β_1-globulins were evident in the secretions of the milked cows about 3 days before parturition. The maximum daily amount of serum albumin also appeared in the secretions about this time.

P26. Preliminary repeatability estimates of per cent total protein and certain other milk constituents of Holstein cows at selected intervals of lactation. S. D. MUSGRAVE, R. E. WALTON, F. A. GRAYBILL, R. MACVICAR, J. B. MICKLE, M. LOEWENSTEIN, and P. E. JOHNSON, Oklahoma Agr. Expt. Sta., Stillwater.

Single-day composite samples of milk were taken for 3 periods of 4 consecutive days each in March, June, and September to obtain preliminary estimates of the repeatability of % total protein (TP), % of fat (F), % total solids by Mojonnier (TS"M") and by Cenco (TS"C") methods, % solids-not-fat (SNF), and lb. of total milk produced (TM), and to obtain estimates of the frequency of sampling necessary to predict protein yield. Winter-calving Holstein cows were used. Estimates of repeatability of daily vs. period variations within months (seasons) were obtained for each cow and then pooled over cows. Variances within cows ascribable to days (σ^2d), periods (σ^2p), and months (σ^2m) were used as follows in obtaining repeatability (R_1, R_2) from analysis of variance data:

$$(1)\quad R_1 = \frac{\sigma^2 d}{\sigma^2 d + \sigma^2 p}$$

$$(2)\quad R_2 = \frac{\sigma^2 d + \sigma^2 p}{\sigma^2 d + \sigma^2 p + \sigma^2 m}$$

The pooled data are as follows:

	%TP	%F	%TS "M"	%TS "C"	%SNF	lb. TM
R_1	0.75	0.84	0.99	0.89	0.96	0.33
R_2	0.60	0.99	0.88	0.80	0.92	0.17

Correlation coefficients between these constituents have also been computed.

P27. Relationship of milk let-down to certain other phenomena of lactation. J. D. DONKER, W. J. MILLER, H. L. DALTON, and I. LEVY, Univ. of Georgia, Athens.

Fifteen cows were milked on the following schedule of between-milking intervals: 11 hours —24 times, 13 hours—48 times, 11 hours—48 times, 13 hours—24 times (6 times at each hour of the day). Period I consisted of the 1st 48 milkings; Period II, the remainder. To measure milk let-down, oxytocin (10 I.U.) was administered to each cow every 3rd and 6th milking in Periods I and II, respectively, to obtain complementary milk. All milks were weighed and all sampled for fat analysis except for milks collected in Period I at times when oxytocin was not used. The effects on complementary milk of hour of day, milking interval, and scheme of injecting oxytocin were considered. Complementary milk (%) was higher ($P = 0.01$) in Period I (11 hr.—13.0%; 13 hr. —12.5%), when oxytocin injections were closely spaced, than in Period II (11 hr.—10.8%; 13 hr.—10.0%). Differences in complementary milk (%) between 11- and 13-hr. milking schedules were not significant statistically. Yield of milk per hour and butterfat content were higher ($P = 0.01$) on the 11-hr. schedule. There were highly significant differences in complementary milk (%) among hours of the day.

P28. Effect of vacuum and pulsation rate on rate of milking.[1] W. E. STEWART and L. H. SCHULTZ, Cornell Univ., Ithaca, N. Y.

Eighteen randomly selected slow-milking cows were milked with a machine operated at 3 vacuum levels (10, 12.5, and 15) and 3 pulsation rates (20, 50, and 80). The experimental design was a 3 × 3 factorial with three replications. Therefore, each cow was milked 3 times at each of the 9 possible vacuum-pulsation combinations.

Rate-of-milking measurements were made by suspending the milker pail from a scale and taking readings every 15 sec. Machine stripping was started when the rate of flow declined to a level of 0.3 lb. or less between readings. The average yield per milking was 20.7 lb.

As the vacuum increased, total milking time decreased ($P < 0.01$). It decreased from 437 sec. at 10 in. to 362 at 12.5 to 328 at 15. Maximum rate of flow increased ($P < 0.01$) from 4.2 lb. per min. at 10 in. to 5.2 at 12.5 to 5.8 at 15. Average rate of flow increased ($P < 0.01$) from 2.9 lb. per min. at 10 to 3.5 at 12.5 to 4.0 at 15.

As the pulsation rate increased, total milking time decreased ($P < 0.01$). It decreased from 405 sec. at 20 to 363 at 50 to 358 at 80. Maximum rate of flow increased ($P < 0.01$) from 4.7 lb. per min. at 20 to 5.2 at 50 to 5.2 at 80. Average rate of flow increased ($P < 0.01$) from 3.3 lb. per min. at 20 to 3.5 at 50 to 3.6 at 80.

Most of the effects of vacuum level and pulsation rate on increasing milking rate were additive.

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[1] Supported in part by the DeLaval Separator Company, Poughkeepsie, N. Y.

P29. Effect of variants in pipeline installations on the acid degree of milk. LOUIS JOKAY and J. M. JENSEN, Michigan Agr. Expt. Sta., East Lansing.

Farm pipeline installations with and without risers and of varying length were observed for their contribution to rancidity in milk as measured by acid degree values, surface tension, and taste. The acid degree was determined by the solvent extraction procedure. The results support previous studies that have shown rancidity to be initiated principally by conditions that cause foaming of milk when fat is in the liquid state.

Acid degree values in milk increased with increase in length of line and increase in number and height of risers. Foaming due to leaky milk valves was a major contribution to high acid degree values. Churning occurred to a serious extent in long milk lines with numerous risers. Acid degree values of high order in milk were brought into normal range by reducing the number and height of risers, the length of lines, and especially the air inlet.

A relationship is shown between taste, surface tension, and acid degree values.

P30. The resistance of milk samples to the activity of mastitis staphylococci. W. D. POUNDEN and NORMA A. FRANK, Ohio Agr. Expt. Sta., Wooster.

Efforts have been made to find ways of measuring resistance of cows to mastitis-producing bacteria. Previous reports have concerned a measure of the relative ability of milk samples to resist acid production by *S. agalactiae*. The present one concerns the ability of samples to resist the activity of staphylococci.

Aseptically collected milk samples to which methylene blue had been added were inoculated with a mastitis-producing strain of hemolytic staphylococci. The hours of incubation at 37° C. required to produce the beginning of a visible color change from blue to white were recorded.

A trend was observed for the relative resistance of weekly collected milk samples from 10 cows to be lower, as shown by shorter incubation periods, from April to July inclusive, compared to later months in both 1954 and 1955. Also, in both years there was a marked shortening in the required length of the incubation period in samples collected in the 2 wk. immediately after the animals went on pasture.

P31. Comparison of antibacterial activity of mastitis products by an in vitro method. K. E. PRICE, Z. ZOLLI, M. J. GALLIAN, and G. SHEA, Agr. Research and Development Dept., Chas. Pfizer & Co., Inc., Terre Haute, Ind.

It has been shown previously that minimum inhibitory concentrations and minimum lethal concentrations of some antibiotics are markedly higher in skimmilk and whole milk than in nutrient broth. The present study compares antibacterial activity in milk and broth of 14 commercial mastitis preparations containing single or mixed antibiotics or other drugs. Flasks containing 2% (wt. or vol.) of each preparation and 50 ml. of milk or broth, were placed on an Eberbach shaker. Samples removed at 5 min. and at 3, 18, and 24 hr. were assayed for total antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, and *Micrococcus pyogenes* strains.

Products containing penicillin, tetracycline, oxytetracycline, chlortetracycline, or combinations of these antibiotics were the most effective in milk against streptococci and micrococci. With *Pseudomonas* and *Escherichia* strains, only a small number of products produced demonstrable inhibition in milk. Preparations containing both oxytetracycline and polymyxin B were most active; those containing chlortetracycline, streptomycin, or mercury showed some activity; and those containing nitrofurazone, sulfonamides, or tyrothricin were essentially inactive. Minimum inhibitory concentrations of sulfas and nitrofurazone (not in dosage form) were higher in milk than in broth for 1 or more test organisms. For most dosage forms, type of vehicle had little influence on activity.

P32. The development of rumen microorganisms in inoculated vs. isolated growing calves. M. P. BRYANT and NOLA SMALL, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Rumen contents from 3 calves inoculated with fresh rumen contents and from 3 calves that had been isolated to prevent gross transfer of rumen microorganisms from mature animals were studied by direct microscopic and by cultural methods. Many of the kinds of bacteria and the full complement of protozoa that predominate in mature cattle became established in inoculated calves at an early age (6-9 wk.). Few of the bacteria predominating in mature cattle and none of the protozoa were found in

the isolated calves even after 17 wk. of age. Also, total anaerobic colony counts tended to be higher in the isolated calves at 6-17 wk. of age. After 2 of the isolated calves were inoculated, there was a drop in total anaerobic counts, and the full complement of rumen protozoa and many predominating bacteria typical of mature animals became established.

Comparisons of the present data with data collected previously on calves raised under normal conditions indicate that inoculation of calves hastens the development of rumen protozoa but will not hasten the development of bacteria typical of the mature rumen unless the calves are raised under rather strictly isolated conditions.

P33. Absorption of volatile fatty acids from the reticulo-rumen of young dairy calves. W. P. FLATT,[1] R. G. WARNER, and J. K. LOOSLI, Cornell Univ., Ithaca, N. Y.

The relative rates of disappearance of volatile fatty acids from the reticulo-rumen were determined in 53 absorption studies with 10 fistulated and 3 laparotomized dairy calves. Ten male Holstein calves fistulated at 6 days of age were allotted to dietary treatments of mineralized milk, hay, dry calf starter, or calf starter and hay at a 2:3 ratio. Absorption studies were made when the calves were 3, 5, 7, 11, and 15 wk. of age by introducing a warm equimolar solution (10 g/l) of butyric, propionic, and acetic acids buffered to pH 6.2 into the empty, clean reticulo-rumen and measuring the losses that occurred in 60 min. The order of disappearance from the rumen was butyric > propionic > acetic, on a weight basis, being 2.66, 2.26, and 1.69 g/hr (av. 50 trials), respectively. On a molar basis the rates were almost equal, with losses of 30.2, 30.6, and 28.2 m*M* per hr. of butyric, propionic, and acetic acids.

Absorption from the isolated reticulo-rumens of 3 laparotomized Holstein calves (6, 14, and 20 wk. of age) was 4-5 g. per hr. with the relative rates being butyric > propionic > acetic, on both a weight and molar basis.

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P34. Studies using synthetic milks for dairy calves. J. W. THOMAS and M. OKAMOTO, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

After their second day of life calves have been fed a synthetic milk diet containing casein, cerelose, lactose, lard, minerals, and vitamins. Calves given this diet, which is complete in all known nutrients, have grown at about 85% of the normal rate. Two were kept on the diet for over 18 mo. but growth was very subnormal after 10 mo. of age. Variations in amounts of vitamins A, B, B_6, and B_{12} and magnesium were studied. Calves receiving 5 mg. alpha tocopherol/cwt died in a paralyzed condition at 3 to 5 mo. of age; those receiving 10 mg. died at 155 to 240 days of age. Calves receiving 20 mg. alpha tocopherol/cwt/day had very low plasma levels (< 0.1 mg. %). Seventy mg/cwt gave plasma levels of 0.3 to 0.6 mg. %.

The rate of growth of calves receiving either no vitamin B_6 or no B_{12} was about 50% of normal. Those receiving no B_6 had lowered concentrations of pyridoxine in their blood and urine. Most calves died in an emaciated condition with anorexia. No convulsions were noted. Calves receiving no B_{12} excreted B_{12} in their feces. The amounts were 0.2 to 0.5 γ/g dry matter at 30 to 140 days of age. One calf had some in its liver. Supplementation with B_{12} improved growth.

Daily magnesium intake necessary to maintain normal plasma magnesium levels on this diet was about 0.6 g/cwt up to 90 days of age; 1.2 g/cwt up to 6 mo. of age, and at least 1.8 g/cwt for longer periods of time.

P35. The value of distillers solubles and vitamin B_{12} in milk replacement formulas with and without alfalfa hay for young dairy calves. G. F. FRIES, G. M. WARD, and C. F. HUFFMAN, Michigan Agr. Expt. Sta., East Lansing.

Forty 3-day-old Holstein calves were divided into 5 groups. A limited amount of milk was fed until the 20th day. Milk replacers were fed in gruel form from the 8th through the 30th day and ad lib. in dry form from the 8th through the 60th day. Group I received a milk replacer composed of 47.1 lb. corn, 32.5 lb. soy flour, 10.0 lb. distillers solubles, 1.5 lb. steamed bone meal, 0.5 lb. $CaCO_3$, 0.5 lb. salt, and 1.0 lb. Aurofac D. The diet of Group II was the same as that of Group I plus alfalfa hay ad lib. Group III was fed the same diet as Group I with the distillers solubles replaced by soy flour and corn. Group IV received the same diet as Group III plus alfalfa hay ad lib. Group V was fed the same diet as Group I plus 5 mg. vitamin B_{12} per 100 lb. of milk replacer. Av. daily gains were 0.79, 0.82, 0.67, 0.87, and 0.55 lb. for the 5 groups, respectively. Pounds of feed per lb. of gain were 2.77, 3.10, 2.88, 3.13, and 3.43 for the 5 groups, respectively. The differences in gains and feed efficiency were not statistically significant.

P36. A study of the lipolysis induced in various milks by pregastric esterases obtained from suckling mammals. M. G. FARNHAM and J. H. NELSON, Dairyland Food Labs., Inc., Waukesha, Wisconsin, and A. R. KEMP, Dean Milk Co., Rockford, Illinois.

Certain tongue tissues of the suckling calf, kid, and lamb are known to secrete significant amounts of esterase(s) that split milk fat. These enzymes, which are termed "pregastric esterases," have been isolated in activity-pure preparations.

This investigation compared certain aspects of the lipolysis induced by the esterase(s) from

each species in cow's milk and in milk from the same species. All milks were pasteurized to inactivate milk lipase. Esterase levels were selected which appeared comparable to the secretory power of the suckling mammal. The enzyme units added to each milk were proportional to the fat content of each milk. The inoculated milks were incubated at 40° C. with gentle agitation. Aliquots were withdrawn periodically for pH and titratable acidity determinations. After 4 hr. of incubation, the free fatty acids were analyzed chromatographically.

All 3 esterases were most active in cow's milk. The differences between the activity of the 3 esterases in cow's milk were not pronounced. The activity of kid esterase in goat's milk was slightly less than in cow's milk. The activity of lamb esterase in ewe's milk was very low.

Chromatographic data were obtained on butyric, caproic, caprylic, capric, and higher acids. A modified Ramsey-Patterson technique was used.

P37. The influence of chlortetracycline on whole blood, plasma, and corpuscle glucose relationships in calves fed high roughage pellets. J. H. VANDERSALL, J. W. HIBBS, and H. R. CONRAD, Ohio Agr. Expt. Sta., Wooster.

Twenty Jersey calves were used in a study of the influence of chlortetracycline on whole blood, plasma, and corpuscle glucose (reducing sugar) relationships during the first 16 wk. Whole milk was fed for 7 wk. High roughage pellets (2 parts ground alfalfa hay and 1 part a simple grain mixture) were fed free choice after the 3rd day. Calves in the experimental group were fed pellets containing 20 mg. of chlortetracycline (Aurofac 2A) per lb. during the first 12 wk. Pellets containing no chlortetracycline were fed to both groups after 12 wk. Whole blood glucose, plasma glucose, and hematocrit determinations were made at the same time of day each week. Corpuscle glucose was calculated. As previously observed, the chlortetracycline-fed calves maintained a consistently higher whole blood glucose level. This higher level was found to be the result of increased plasma glucose. No statistically significant difference was found in corpuscle glucose levels between the control and experimental groups. Much of the decline in blood glucose during the first few weeks was due to a decline in corpuscle glucose. A similar observation has been made by R. L. Reid working with lambs.

P38. Effect of chlortetracycline and diethylstilbestrol on growth, carcass, and endocrine glands of dairy calves when fed singly and in combination. BARNEY HARRIS, JR., and L. L. RUSOFF, Louisiana Agr. Expt. Sta., Baton Rouge.

Thirty-two male calves, 24 Holsteins and 8 Jerseys, from the L.S.U. dairy herd were divided at random according to breed and placed into 4 groups: Group I served as the control; Group II received 50-75 mg. of chlortetracycline daily; Group III, 10 mg. of diethylstilbestrol daily; and Group IV, 50-75 mg. of chlortetracycline and 10 mg. of diethylstilbestrol daily. All calves were fed colostrum for the first 3 days, after which they received whole milk at the rate of 10% of body weight up to 30 days of age. A plant protein calf starter and alfalfa hay were fed beginning at 4 days of age. The calf starters for Groups II and III were supplemented with ½% Aurofac 2A. Preliminary results at 16 wk. of age indicate that a growth response was obtained in the groups receiving chlortetracycline. The supplementation of the diet with diethylstilbestrol appeared to have no effect on growth. Only a few cases of scours occurred in this experiment. The weights of the endocrine organs, and carcass analyses, will be presented.

P39. Effects of oral administration of diethylstilbestrol on young dairy calves. H. H. VOELKER and A. E. DRACY, South Dakota State College, Brookings.

Forty-eight calves (Holstein, Brown Swiss, Guernsey; 34 males, 14 females) were used in 3 experiments to determine the responses to feeding diethylstilbestrol (Stilbosol, Lilly Co.) from 4 to 88 and from 4 to 116 days of age. Females were continued on diethylstilbestrol to determine its effects on estrus and reproduction. Calves were individually fed concentrates ad libitum. Two mg. (3 mg. in Expt. III) stilbestrol per 100 lb. body wt. were fed daily in milk to half of the calves; the other calves were controls. Males were slaughtered at 88 days in Expt. I. In Expt. II and III they were castrated at 88 days and stilbestrol was continued through 116 days. Stilbestrol induced no significant effects on wt. gains, body measurements, and feed consumption or utilization. At 88 days stilbestrol-fed groups averaged 197 lb.; controls, 208 lb. At 116 days hormone-treated calves averaged 249 lb.; controls, 261 lb. Dressed % was 59.8%, controls. Testes wt. averaged 9.8 g. in treated calves; 21.07 g., controls. Testes, tunica albuginea, epididymus, and vas deferens averaged 18.9 g. in stilbestrol-fed calves; 38.1 g. in controls. Stilbestrol stimulated rudimentary teat development. Stilbestrol-fed calves were not different in growth of thymus, kidneys, liver, penis, or adrenals. Sex organ microtome and microphotographic sections are presented.

P40. A comparison of three levels and two sources of crude fiber in calf starters. R. T. WHITAKER, W. J. MILLER, H. L. DALTON, and J. L. CARMON, Univ. of Georgia, Athens.

In seven replications, Holstein and Jersey calves were fed 5 relatively simple calf starters. The basal starter contained 5% crude fiber, and the other starters constituted a 2 × 2 factorial design: each contained either 9% or 13% crude

fiber obtained by adding either corn cobs and shucks or alfalfa meal to the basal starter. All starters were adjusted to 16% crude protein by the addition of degossypolized cottonseed meal. The calves were fed a limited amount of whole milk and milk replacer, long alfalfa hay ad lib., and the starters ad lib. up to 4 lb. per day.

There were no statistically significant differences in gains, starter consumption, or hay consumption among the calves fed the various starters to 15 wk. When initial weight and hay consumption were held constant, there was a partial correlation of 0.950 between starter consumption and gain and a partial regression coefficient which indicated an increase in gain of 0.48 lb. for each lb. increase in starter consumption. Similarly, the partial correlation between hay consumption and gain was 0.791 and the partial regression indicated that an increase in hay consumption of 1 lb. resulted in an increase in gain of 0.22 lb.

P41. High-moisture vs. wilted grass silage for raising dairy calves. J. A. Newlander and W. H. Riddell, Univ. of Vermont, Burlington.

The nutritive values of high-moisture grass silage (av. H_2O, 79.26%) and wilted grass silage (av. H_2O, 67.79%) were compared in the raising of dairy calves. Six sets of identical twins were used during 2 silage feeding years, 1953-55. The calves were started on silage at 3 to 4 mo. of age. Their rations consisted of silage ad lib. plus 3 lb. of grain daily the 1st year but only 2 lb. daily the 2nd year.

The calves on the wilted silage gained, during the 1st year (16-wk. trial), 10.70% more than their mates on the wet silage. In the 2nd year (19-wk. trial) the extra gain was 10.92% in favor of the calves fed wilted silage.

Intangible losses of dry matter, as measured by bag samples, showed an average loss for the 2 yr. of 16.24% in the wet silage and 9.23% in the wilted.

P42. The influence of chlorinated napthalenes on the utilization of vitamin A by calves and sheep. R. G. Warner, K. L. McEntee, Wm. Hansel, and C. H. Grippin, Cornell Univ., Ithaca, N. Y.

Nine 4-mo.-old grade Holstein heifers and six 4-mo.-old native ewe lambs were depleted of their vitamin A stores. The animals were considered depleted when the blood plasma vitamin A remained below 4 γ% for 2 consecutive wk. At depletion all animals received sufficient stabilized vitamin A powder 3 times weekly to supply vitamin A at the rate of 21,900 I.U. per 100 lb. body wt. per day. The calves were divided at random into 3 groups of 3 animals each and received highly chlorinated naphthalenes dissolved in corn oil 3 times weekly at the following rates per 100 lb. body wt. per day: none, 1.1 mg., and 5.4 mg. The lambs were divided into 2 groups and received chlorinated naphthalenes at the rate of none and 54 mg. per 100 lb. body wt. per day. The supplements were continued for 3 wk. The animals were then slaughtered and total liver vitamin A was determined. The chlorinated naphthalene dosage, micrograms of liver vitamin A per 100 lb. body wt., and blood plasma vitamin A in micrograms per cent were, respectively, calves: (none) 108 $\pm$ 7.7, 35.9 $\pm$ 3.1; (1.1 mg.) 36.4 $\pm$ 12.9, 29.3 $\pm$ 1.7; (5.4 mg.) 18.6 $\pm$ 2.9, 15.3 $\pm$ 5.3; lambs: (none) 250 $\pm$ 14.7, 42.9 $\pm$ 12.3; (5.4 mg.) 116 $\pm$ 13.6, 49.5 $\pm$ 9.4 Lesions indicative of chlorinated naphthalene poisoning were observed in all animals that had received the supplement.

P43. Relation of various nutritional factors to diarrhea in the young calf. F. G. Owen, N. L. Jacobson, and R. S. Allen, Iowa State College, Ames.

The influence on incidence of diarrhea of alterations in the normal composition of milk diets was evaluated with calves (4 to 90 days of age) kept in pens equipped with metal screen bottoms. Dietary fat, lactose, minerals, and curd formation were tested, individually and in all possible combinations. Incidence and severity of diarrhea were not influenced by prevention of curd formation through the addition of sodium citrate. However, diarrhea was reduced by inclusion of milk fat (3%), was increased slightly by doubling the lactose (2×), and was increased moderately by increasing the mineral content of the milk to 4 times the normal amount by addition of a simulated milk ash. Other factors also apparently are involved in the response to minerals, since in further studies made with a slightly different feeding and management regime, the mineral content of whole milk rations was increased to 10 times the normal amount with no appreciable increase in diarrhea.

In further studies the qualitative and quantitative characteristics of contents of various segments of the digestive tract of calves 14 hr. after feeding a diarrheic type of diet (raw skimmilk plus lactose, 2×, and minerals, 4×) were compared to those of calves fed whole milk. Analytical data will be presented.

P44. Chest girth–weight relationships for Holstein, Jersey, Guernsey, and Ayrshire males. H. P. Davis, Univ. of Nebraska, Lincoln.

As a result of measurements taken during a long-time growth study at the Univ. of Nebraska, data were available for a study of the chest girth–weight relationship for males of the 4 dairy breeds. The age period commenced at birth and lasted as long as the males remained in the herd. Most of the data covered the period from birth to 18 mo. The ranges in chest girth in cm. for which weight rela-

tionships were available were as follows: Holsteins, 61-214; Jerseys, 55-160; Guernseys, 67-136; and Ayrshires, 65-171. Tables will be presented showing the weight relationship for each chest girth for each breed, smoothed by calculating a moving average. On the basis of the weight–chest girth relationship for each month of age, birth to disposal, the correlations for the several breeds and the pooled data for all breeds were: Holsteins, 0.9871; Jerseys, 0.9840; Guernseys, 0.9850; Ayrshires, 0.9872; and all breeds, 0.9796. Because of these relationships and other unpublished data, a chest girth–weight relationship table for all breeds will be presented.

P45. Factors affecting birth weight of calves in a crossbreeding experiment. T. G. MARTIN, Purdue Univ., Lafayette, Ind.

In the past 4 yr. 178 normal-term, single-birth calves have been borne by foundation cows of the Red Dane, Red Poll, and Milking Shorthorn breeds. There were 103 male and 75 female calves. The Red Dane, Red Poll, and Milking Shorthorn bulls, respectively, sired 55, 16, and 11 calves from Red Dane cows; 16, 28, and 8 calves from Red Poll cows; and 11, 11, and 21 calves from Shorthorn cows. An analysis of variance of the birth weights showed that male calves were 4.8 lb. heavier than females and that birth weight increased steadily from first to fifth calving with fifth calves being 11.4 lb. heavier than first calves. Red Dane cows gave birth to calves 6.1 and 7.3 lb. heavier than calves from Red Poll and Shorthorn cows, respectively. Differences between sires within breeds were as large as differences between breeds. Calves sired by Red Dane bulls were 8.0 and 5.8 lb. heavier than calves sired by Red Poll and Shorthorn bulls, respectively. Crossbred calves averaged 1.9 lb. heavier than purebred calves. Sex, order of calving, breed of sire, sire in breed, and breed of dam were significant ($P \leq 0.05$) sources of variation.

P46. A study of sire influence on rate of maturity in several Holstein-Friesian families. H. L. BARR, T. M. LUDWICK, and FORDYCE ELY. The Ohio State Univ., Columbus, and Ohio Agr. Expt. Sta., Wooster, in cooperation with The Dairy Husbandry Research Branch, USDA.

The daughters of 8 sires in 1 herd were used to study maturity rates as indicated by production. A maturity index was computed for each daughter by dividing the second record by the record made at first calving, both converted to mature equivalent. The means of indexes for the 8 sire groups ranged from 1.024 to 0.851.

Of the 28 comparisons possible among the 8 sire groups, 6 were found to be significantly different at the 5% level. All of the comparisons in which differences were found would have shown larger differences had correction factors for dry period, calving interval, and season of calving been used. Other environmental factors were found to be virtually constant among the groups involved in 2 of the 6 aforementioned comparisons. Some of the differences in the remaining 4 comparisons may have been caused by differences in the time at which various groups made their records.

A study of growth rates of the sire groups involved in 1 comparison indicated that the group which matured late, as measured by the maturity index, also reached maximum size at an older age.

P47. The reliability of some intra-herd production information used in selecting sires for use in artificial insemination. H. W. CARTER, R. ALBRECTSEN, C. R. HENDERSON, and J. T. GODFREY, Cornell Univ., Ithaca, N. Y.

This study was made to determine what information on the production of a sire's natural service daughters in a single herd is more dependable than the daughter-dam comparison in determining the production of his artificially sired daughters. Available were 21 Holstein sires with 50 or more tested daughters resulting from artificial insemination and for which complete information was available on their natural service daughters.

The regression of the information being studied on the artificially sired daughters' average was obtained. Regression of daughter-dam difference on A. B. daughter average equals 0.2001.* Regression of natural service daughters' average on A.B. daughter average equals 0.0013. Regression of equal parent index on A.B. daughter level equals 0.1980.* Regression of natural service average contemporaries' difference on A.B. daughter level equals 0.2023.* Regression of difference between natural service daughter average and dams' predicted transmitting ability on A.B. daughter level equals 0.2633.*

From these regression factors it appears that the difference between the production of a sire's natural service daughters and their contemporaries was equal to, but no better than, daughter-dam comparisons for use in selecting sires to use in artificial insemination. Daughter average is of little or no use and equal parent index slightly less dependable than daughter-dam comparisons.

* Significant at the 5% level.

P48. Estimates of genetic progress in the development of the American Red Danish cattle. N. R. THOMPSON and N. P. RALSTON, Michigan State Univ., East Lansing.

Milk and butterfat yields and related information were collected on the foundation generation and on successive back-crosses to Red Danish sires. Least squares estimates revealed that butterfat yield did not vary significantly with month of calving but did vary significantly ($P < 0.01$) with age at calving, previous calv-

ing interval (2.0 lb. per mo.), and length of lactation period (1.0 lb. per day). An upward environmental trend of 6.5 lb. per year ($P < 0.05$) was found.

After adjustment for significant effects, the foundation, first cross, second cross, and third cross generations averaged 351, 379, 377, and 389 lb. butterfat, respectively. Gains from foundation to first cross and from second to third cross were highly significant ($P < 0.001$).

Selection gave estimated additively genetic superiorities of 0 to +13.4 lb. butterfat in the foundation generation, and 0 to +5.1 lb. in the first and second cross generations. Heritability of butterfat yield was estimated by intrasire regression of daughter on dam as 0.39 ± 0.11; repeatability, as 0.43. Correlation of age at calving and calving interval was 0.07 ($P < 0.05$).

P49. Relations between monthly test-day milk production of Holstein-Friesian cows.[1] D. E. MADDEN, L. D. MCGILLIARD, and N. P. RALSTON, Michigan State Univ., East Lansing.

Milk production records for the first 10 monthly test days were obtained from 6,495 Holstein-Friesian H.I.R. records. Factors for extending part records to a 305-day basis were obtained by the ratio of the sum of the milk production for the first 10 monthly test days to the part record and are:

	Milking frequency					
	2×			3×		
	Age fresh					
No. of test days	All ages	2 years	6 years	All ages	2 years	6 years
	No. of records					
	5,501	1,492	493	994	245	100
1	7.64	8.36	7.45	8.06	8.69	8.21
2	3.81	4.15	3.69	3.98	4.32	3.99
3	2.60	2.82	2.53	2.69	2.91	2.69
4	2.02	2.16	1.96	2.08	2.22	2.07
5	1.67	1.77	1.63	1.71	1.81	1.71
6	1.44	1.51	1.41	1.47	1.54	1.47
7	1.28	1.32	1.26	1.30	1.34	1.29
8	1.16	1.18	1.14	1.17	1.20	1.17
9	1.07	1.08	1.06	1.07	1.08	1.07
10	1.00	1.00	1.00	1.00	1.00	1.00

The factors for 2×—all ages, are comparable to those presented in the literature. The larger factors for the records initiated at 2 yr., when compared with those for 6 yr., indicate the greater persistency of the first lactation. The factors for 3× illustrate the greater persistency of these records when compared with 2× records, as well as showing an age effect similar to the 2× records.

[1] Records furnished by Holstein-Friesian Association of America, Brattleboro, Vt.

Other relations were considered, including relations between test-day production and 305-day production as computed by the "calendar" method, and relations between production from various numbers of test days.

P50. Causes of variation in calving interval of Holstein-Friesian cows. J. C. RENNIE,[1] Iowa State College, Ames.

A total of 1,663 calving intervals (731 cows) from 3 herds over an 11-yr. period (1940-51) was studied. The modal class was 360-380 days, the average was 413 days, and the standard deviation was 78 days. The intra-herd repeatability corrected for yearly differences was 0.184. Yearly differences accounted for only 4.8% of the intra-herd variance in individual calving intervals. Heritability of differences in length of calving interval was estimated as 0.03 ± 0.11 by doubling the intra-sire regression within herd of daughter on dam and as 0.148 by the paternal half-sister correlation method.

The intra-herd and year linear regressions of milk and fat production on length of current calving interval were 73.5 lb. of milk and 2.38 lb. of fat, per 10-days' increase. The regressions of milk and fat on preceding calving interval (intra-herd) were 60.9 lb. of milk and 2.30 lb. of fat, per 10-days' increase. These regressions measured only the linear association between length of calving interval and production; however, it appeared that a curvilinear association existed for calving intervals shorter than 400 days.

The records of 222 cows were used in studying the influence of management on length of calving interval. On the average, the first observed estrus occurred 55 days after parturition, with a standard deviation of 29 days. An average interval of 26 days elapsed from the time of first estrus until first breeding.

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P51. Interrelationships between productivity and gestation lengths in dairy cattle. R. C. LABEN, W. C. ROLLINS, and S. W. MEAD, Univ. of California, Davis.

Records of the University Jersey herd show a highly significant apparent effect of age of dam on gestation length. First gestations were 1.3 days shorter than second, and second, 1.2 days shorter than third, after which no definite trend was observed. First and second gestations proceeded on a high nutrient plane and later gestations on a lower plane. Restricted feed intake in horses lengthens their gestation period. These observations suggest that age effects after the first gestation in dairy cows might be due more to management than to physiological age. Pregnant heifers lack the lactation stress normally found during most later gestations. The relationship between feeding level and gestation length suggests that in-

creased length is a symptom of stress in the cow and led to this hypothesis: Cows with high milk yield genotypes might be under greater internal stress than low producers and therefore exhibit an increased length of reproductive cycle. First lactation FCM yield increased second gestation length 0.03 day per 100 lb. production increase. The regression, significant at the 5% level, approaches the 1% level when corrected for inbreeding. Also, higher producers tended to take slightly longer to settle. These and other findings agree with the hypothesis developed from this preliminary study.

P52. The influence of season of freshening on production records of Jersey and Holstein cows in Louisiana. J. E. JOHNSTON, CLYDE LEWIS, E. J. STONE, and CECIL BRANTON, Louisiana Agr. Expt. Sta., Baton Rouge.

The influence of season of freshening on 2 × 305 M. E. production of FCM was studied for a 10-yr. period in the LSU Jersey and Holstein herds. The study included 120 Jerseys and 168 Holsteins which had a total of 353 and 524 production records, respectively. The seasons studied were Jan.-March, April-June, July-Sept., and Oct.-Dec. Fewer cows freshened during the April-June season than during other seasons. Year-to-year differences were marked and highly significant in both breeds. Jerseys made highest records when they freshened during the Jan.-March season, with mean of 8,339 lb. FCM, and lowest records when they freshened during the July-Sept. season, with mean of 7,444 lb. FCM. Seasonal differences in the Jersey breed were highly significant and no season-year interaction could be demonstrated. Holsteins showed highly significant season-year interaction, so analysis was carried out by using weighted means. Seasonal differences were not statistically significant; mean production for Jan.-March freshenings was 9,654 lb. FCM, and for April-June freshenings was 9,207 lb. Breed differences may have been due to greater sensitivity of Holsteins to unfavorable climatic and nutritional conditions, which vary markedly from year to year.

P53. Mammary gland measurements in calves and their prediction value in herds of varying environment.[1] V. L. BALDWIN,[2] W. E. PETERSEN, and N. D. BAYLEY,[3] Univ. of Minnesota, St. Paul.

Of 927 calves on which the mammary glands were measured, production records were available for 262 Holsteins in 90 herds from 69 sires, and for 105 Guernseys, Jerseys, Brown Swiss, and Shorthorns in 40 herds from 46 sires. Analysis of variance and correlation analysis revealed poor predictability of milk or fat production from palpation grade in Holstein calves from 6 levels of calf management or nutrition. Average production of daughters of individual sires was also not predicted accurately.

Calf management was shown to be an important factor in the relationship. The r^2 indicated that 72% of the variation in milk production from 1 management grade to another is associated with variation in palpation grades from 1 management grade to another. Possibility of greater prediction values in better managed herds was indicated when, within sires, the correlation coefficients increased with increasing management grades from −0.03 to +0.29. Numbers were small and the r's were not significant.

Incomplete information as well as disease, breed, and year influences on udder palpation grades and production appeared to have affected the data on breeds other than Holstein and produced erratic estimates of correlation.

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[1] This work was part of a Ph.D. thesis at the Univ. of Minnesota.

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P54. Withdrawn

P55. The influence of heredity and environment on the fertility of dairy cattle. CECIL BRANTON, W. S. GRIFFITH, H. W. NORTON, and J. G. HALL, Louisiana Agr. Expt. Sta., Baton Rouge.

The hereditary and environmental aspects of the fertility of dairy cattle have been investigated in 2 experiments. In the first experiment the breeding and calving records of the LSU Holstein herd from 1931 through 1946 were analyzed. These data included 381 cows representing offspring of 15 sires. Estimates of the repeatability and heritability of services per conception and the influence of postpartum interval, age of cow, and season of the year on fertility were determined. In the second experiment feeding, management, and breeding practices and diseases were related to the fertility of cows in 85 herds located in various sections of Louisiana. A total of 383 problem cows were palpated rectally and checked for genital diseases in these herds.

The estimates of heritability and repeatability of services per conception were 0.097 and 0.106, respectively. The postpartum interval to breeding, the season of the year, and genital diseases such as brucellosis and vibriosis were found to influence fertility markedly. Age of cows appeared to have no influence. The remainder of the data is being analyzed.

P56. The amount of pigmented body area in spotted cattle as associated with head pattern and as influenced by sex. J. M. TREECE, L. O. GILMORE, R. LAIRD, and N. S. FECHHEIMER, Ohio Agr. Expt. Sta., Wooster and Columbus.

Photographs of 327 Holstein females and 206 Holstein males, taken during first 6 mo. of age, were used to study the effect of sex on body

pigmentation (area) and the relation of the amount of body pigment area to the head pattern. The repeatability calculated from ratings made on the amount of body pigment at 3 widely separated intervals of time was 99%. Females had approximately 6 percentage units more pigmented area on the body than males (highly significant).

The amount of pigment on the body was found for each head pattern group, i.e., whitehead, blaze, shield and snip, and completely pigmented head. These values with corresponding standard errors were, respectively: 21.6 ± 1.9, 40.5 ± 1.5, 61.8 ± 2.0, 79.7 ± 1.2, and 90.7 ± 1.4.

When body pattern was disregarded in these data, no mating type for head pattern was found to breed true. However, there were no pigmented head × pigmented head matings. The head pattern distribution of 121 offspring of whitehead × nonwhitehead matings supports the conclusion that whitehead pattern is due to a dominant gene.

P57. Progress report—Studies of total milk solids as related to inheritance. H. S. Willard, W. R. Thomas, and D. C. Brown, Wyoming Agr. Expt. Sta., Laramie.

There is a growing trend in many areas to pay for milk on a total-solids rather than butterfat basis. An experiment was designed to determine if the ability to produce milk of high solids content is inherited by the dairy cow.

Holstein cows from the Wyoming Univ. dairy were used for the experiment. The cows for study were selected according to sire and daughter-dam groups. Over a period of 2 yr. milk samples from each cow were taken both morning and night during the entire lactation period and tested for butterfat and for total solids.

Statistical data for 3 sire groups and approximately 30 cows are presented to show the relationship to inheritance of solids-not-fat and of total solids.

P58. Peripheral leucocytic picture in ketotic cows. G. S. Bajwa, R. A. Gessert, and A. C. Chung, Univ. of Maryland, College Park.

Ketotic cows in the Maryland area at various stages of the condition were observed in an attempt to correlate the peripheral leucocytic picture with the stage of ketosis and to evaluate the usefulness of these observations in clinical medicine. Blood samples from 43 ketotic cows were subjected to both total leucocyte count and differential count. In the majority of the cases the total leucocyte count varied from 5,000 to 10,000 cells per cu. mm. of blood, only 6 cows exhibiting counts over 10,000 and only 8 below 5,000; the average was approximately 7,000 cells per cu. mm. Great variations in the levels of eosinophils were noticed, ranging from 0 to 1,900 eosinophils per cu. mm. of blood, with the average approximately 470 per cu. mm. The majority of the cases showed relatively low eosinophil levels. The peripheral leucocytic picture in the ketotic cows did not appear to follow a regular pattern but did fall into 3 main categories: *(a)* showing eosinopenia, neutrophilia, and lymphopenia; *(b)* showing eosinopenia, neutrophilia, and lymphocytosis; and *(c)* showing eosinophilia or normal eosinophils, neutropenia, and lymphocytosis. A marked increase in the levels of nonsegmented neutrophils (immature forms) was almost invariably observed. These marked variations in the peripheral leucocytic picture are believed to be dependent upon the integrity and activity of the pituitary-adrenocortical system.

P59. Adrenal cortical atrophy induced on goats by per os administration of 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane (DDD or TDE). B. C. Hatziolos, Univ. of Maryland, College Park.

Daily per os administration of small doses of DDD (80-200 mg/kg) to young goats resulted in atrophy of the zona fasciculata and the zona reticularis within 1 to 2 mo. At an early stage, the cells showed a progressive lipoid accumulation followed by individual or small-group cell degeneration, pyknosis, and necrosis. Simultaneously, scattered hemorrhages and mild, diffused, polymorphonuclear and plasma cell-infiltration were occasionally noted. The reticular and vascular framework was less affected. At an advanced stage, foci of lymphocytic infiltration appeared, replacing the destroyed epithelial cells of the zones. At a late stage, despite limited changes in size and weight of the adrenals, both zones were replaced by lymphoid, adipose, and fibrous tissue, completely changing the normal structure of the cortex. In many places these lesions extended into the zona glomerulosa, which in some cases was noticeably enlarged. Qualitative changes of the lipoid material also occurred. The fat droplets showed negative Schultz and plasmal reaction. With the onset of adrenal insufficiency, certain manifestations were observed, including rough hair, poor and capricious appetite, staggering gait, and dull expression of the eyes. Cortisone, injected intramuscularly, gave dramatic, but temporary, results.

P60. The effect of cobaltous chloride administration on blood sugar and on alpha cells of the pancreatic islets of goats. B. C. Hatziolos, Univ. of Maryland, College Park.

Intravenous or subcutaneous injections of 15-30 mg/kg of $CoCl_2$ to which hyaluronidase had been added caused a marked transient hyperglycemia (200-300% increase) of about 4-hr. duration in normal goats. Repeated doses of the same amounts resulted in duplication of the reaction. In alloxan-diabetic goats, $CoCl_2$ caused a similar transient blood sugar increase, although it was relatively less pronounced than in normal goats. $CoCl_2$ injections also produced a selective destruction of the pancreatic alpha

cells—the alleged producers of the hyperglycemic factor (HGF)—by autolysis. However, this destruction was by no means universal. Persistence and new formation of alpha cells from ductal epithelium were evident. Even with marked alpha-cell damage, hyperglycemia in normal goats and aggravation of diabetes in alloxanized goats occurred. Simultaneously with alpha-cell damage, severe changes with marked glycogen depletion in the liver and hemorrhages in the adrenal medulla were noted. The use of dehydroergotamine (DHE), the adrenergic blocking drug, reduced the blood glucose reaction by about 40%. The above leads to the conclusion that the hyperglycemia elicited by $CoCl_2$ is mediated in various ways, one of which is stimulation of the sympathetic nervous system.

P61. Bromsulphalein elimination rate in dairy cattle with simultaneous determination of liver function and blood and plasma volumes. J. P. MIXNER and W. G. ROBERTSON, New Jersey Agr. Expt. Sta., Sussex.

A true first-order reaction expressed the manner in which bromsulphalein (2-5 mg. per kg. body wt.) was eliminated from the plasma of dairy cows and bull calves, in which the log of dye concentration was related in a linear manner to time after dye administration. In 10 lactating cows and 7 bull calves the mean dye elimination rate per minute was 19.8% and 15.2%, respectively. By using standard dye dilution techniques with bromsulphalein, mean total plasma volumes in the cows and calves were found to be 21,224 ml. and 3,265 ml., and plasma volumes per kg. of body wt. were 38.1 ml. and 80.6 ml., respectively. Similarly, mean total blood volumes in the cows and calves were 31,831 ml. and 5,582 ml., and blood volumes per kg. of body wt. were 57.2 ml. and 136.8 ml., respectively. Dye elimination rate is superior to rate of plasma clearance per min. per kg. of body wt. (Goodman, *J. Lab. Clin. Med.*, **40**: 531. 1952) in evaluating liver function, since the latter rate is markedly affected by stage of maturity and degree of fatness of the animal and by plasma volume per se.

P62. Determination of liver function and plasma and blood volumes in ketotic cows by using bromsulphalein. W. G. ROBERTSON, W. W. BAILEY, H. D. LENNON, JR., and J. P. MIXNER, New Jersey Agr. Expt. Sta., Sussex.

Robertson *et al.* (*J. Dairy Sci.*, **38**: 611. 1955) reported that ketotic cows exhibited high blood 17-hydroxycorticosteroids and low PBI, suggesting that this may be a reflection of a relative adrenal insufficiency induced by hypothyroidism. The high blood steroids could represent low tissue utilization or decreased liver function (elimination) or a combination of both. Ketosis and the associated hypoglycemia may itself be caused by liver dysfunction, which would partly be expressed as depressed gluconeogenesis and a failure to respond to circulating glucocorticoids under the hypothyroid state. Liver function in 5 normal and 6 ketotic cows was assessed by the bromsulphalein (BSP) method of Mixner and Robertson. The mean BSP elimination rate for the normal and ketotic cows was 19.2% and 9.1%, respectively. Liver function returned toward normal as the animals recovered from ketosis. The hematocrits, plasma, and blood volumes per kg. of body wt., for the normal and ketotic cows, respectively, were 35.6 and 37.0%; 38.1 and 47.8 ml.; and 57.2 and 73.1 ml. These results are in marked contrast to the results of Vigue (*J. Am. Vet. Med. Assoc.*, **127**: 101. 1955), who reported high hematocrits and low blood volumes per kg. of body wt. in ketotic cows.

P63. Some results of parathyroidectomy of ruminants. V. R. SMITH and G. H. STOTT, Univ. of Wisconsin, Madison.

The parathyroid glands were removed from calves of various ages, from pregnant goats, and from pregnant cows. Serum calcium and inorganic phosphorus levels were observed before and after parathyroidectomy. A precipitous decrease in serum calcium occurred within 24 hr. after parathyroidectomy. The immediate post-operative level of calcium was approximately half that of the pre-operative level. Generally, the plasma inorganic phosphorus level decreased in mature animals and increased in young animals. Parathyroidectomy of young calves resulted in tetany and death within a few days if calcium salts were not administered. Several weeks after parathyroidectomy the serum calcium returned to approximately normal levels after the initial drop in some of the older ruminating calves. Goats 4 mo. pregnant aborted as the result of tetany within a few days after parathyroidectomy. Tetany has not been observed in pregnant cows that were parathyroidectomized, although the post-operative level of calcium was about half of the pre-operative level.

P64. A cyto-physiological study of the bovine parathyroid glands. G. H. STOTT and V. R. SMITH, Univ. of Wisconsin, Madison.

Parathyroids from bovines in various physiological stages were studied. Stains specific for lipichondria (Golgi apparatus) were used. For the purpose of this study, size and number of lipichondria were used as criteria of function. The results show a marked increase in number and size of lipichondria from the dry cow, through parturition and early lactation, to a maximum development in the peak of lactation. Sections of parathyroids from calves on milk indicated little activity.

P65. Evaporative cooling in Indian- and European-evolved cattle.[1] R. G. YECK and SAMUEL BRODY, USDA and Univ. of Missouri, Columbia.

Total amount of vaporization was measured in quietly standing lactating cows and in 5- to

15-mo.-old heifers. Evaporative cooling in Cal/m^2/day increased in European cows from 1,000 at 50° F. (23% of heat production) to 2,900 at 105° F. (104% of heat production); in Indian cows from 800 at 50° F. (29% of heat production) to 2,400 at 105° F. (112% of heat production); in lightly clothed sitting man (Gagge *et al.*) from 240 at 50° F. (18% of heat production) to 2,500 at 105° F. (200% of heat production). In heifers at 50° F. Shorthorns vaporized 1⅔ and Santa Gertrudis 1⅓ times as much as Brahmans; at 80° F. the respective figures were 1½ for Shorthorns and 1¼ for Santa Gertrudis. From 50° to 80° F. Brahman heifers' vaporization rates increased 267%, Santa Gertrudis', 250%, and Shorthorns', 200%. It was concluded that total vaporization in Cal/m^2 is less in Indian than in European cattle, and still less in man. Rise in vaporization with increasing temperature above 80° F. is, however, very much greater in man than in cattle and somewhat greater in Indian than in European cattle. Slides will illustrate data.

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[1] Contribution from the Mo. Agr. Expt. Sta., Journal Series No. 1612. Approved by the Director.

P66. Estimation of dry matter digestibility by employing randomly procured samples of feces. W. A. HARDISON, W. N. LINKOUS, and C. Y. WARD, Virginia Agr. Expt. Sta., Blacksburg.

Eight Holstein heifers were divided into 2 comparable groups on the basis of age and wt. One group was fed the top portion of the alfalfa plant and the other group the bottom portion of the plant. The forage was cut in the pre-bloom stage and fed fresh twice daily in amounts which were readily consumed by the animals. Feces were totally collected over a 7-day period. In addition, partial fecal samples were collected from each animal at 6 A.M., 12 noon, 6 P.M., and 12 midnight on each of the 7 days. Dry matter digestibility estimates determined from these partial fecal samples by the plant chromogen technique compared favorably with those determined by the total collection method (70.1 vs. 70.2). Digestion coefficients estimated from fecal samples procured at various times did not differ significantly. The data demonstrate that D.M. digestibility estimates can be made accurately from the analysis of small grab samples collected almost any time during a 24-hr. period. The bottom portion of the alfalfa plant was more variable in digestibility than the top portion.

P67. The utilization of a mixture of alfalfa (Medicago falcata) and smooth bromegrass (Bromus inermis) under rotational and strip grazing systems of pasture management. A. L. BRUNDAGE and W. J. SWEETMAN, Alaska Agr. Expt. Sta., Palmer.

Approximately 7 acres of alfalfa-bromegrass pasture were divided into 2 equal areas in 1955. One area was further subdivided into 3 paddocks which were rotationally grazed, and the other was strip-grazed by means of temporary fences moved daily. Four matched pairs of dairy cows, divided at random into 2 groups, served as 4 replications of the experiment in lieu of replicated pastures. The entire pasture was grazed 3 consecutive times in June and July and a fourth time in late August and early Sept. for a total of 76 days. Calculated TDN yields were 7,145 lb. under strip grazing and 5,873 lb. under rotational grazing. This difference was highly significant ($P < 0.01$).

P68. Progress report on soilage feeding of dairy cattle. B. W. HENDERSON, J. W. COBBLE, and H. J. COOK, Univ. of Rhode Island, Kingston.

Studies on a soilage feeding program have been conducted with 12 pairs of lactating cows. Production of FCM per cow was 3,332 lb. for the soilage group and 3,614 lb. for the pasture group. Milk production per acre was 1,960 lb. per cow for the soilage group and 1,160 lb. per cow for the control group. The area required per cow was 1.7 acres for the soilage cows and 3.1 acres for the pasture cows. Consumption varied from 72 to 278 lb. per cow per day with a mean of 125.5 lb., or 11.1 lb. per 100 lb. of body wt. Changes in body weights were insignificant.

A study was conducted with a limited number of animals on the effect of alternating cows between pasture and soilage. There were no significant differences in production when cows were on soilage for 5 days and pastured for 2 days, each wk., for a period of 7 wk.

In a study with dairy heifers the soilage group gained 1.38 lb. per day and the pasture group gained 1.58 lb. per day. The consumption of green feed ranged from 48 lb. to 247 lb. with a mean of 126.5 lb., or 14.7 lb. per 100 lb. of body wt.

P69. A comparison of summer annuals for milking cows. B. F. HOLLON, W. W. WOODHOUSE, and R. D. MOCHRIE, USDA and North Carolina State College, Willard and Raleigh.

Twelve acres were seeded in May, 1954 to 3 replicates of common Pearl millet and Starr millet *(Pennisetum glaucum)* and sweet Sudan grass *(Sorgum sudanense)* at the Coastal Plain Research Station, Willard, N. C. Eighteen milking Jerseys were used as testers and "put-and-take" heifers were added to remove excess forage when necessary. The calculated mean TDN yields per acre for the season were, for Starr and Pearl millet and Sudan grass respectively, 2,080, 1,554, and 785 lb., with S.E. 345. The good regrowth of the millets accounted for their higher productivity. Starr and Pearl provided some grazing for 10 and 8 wk., respectively, whereas Sudan was grazed

for only 6 wk. Of the total TDN produced, 67% was removed from the Sudan plots in the first 2 wk. The yield from the millets was distributed more uniformly over the grazing period. The total FCM obtained per acre for Starr and Pearl millet and Sudan grass was 2,543, 2,181, and 1,169 lb., respectively.

P70. The effect of fine grinding of hay on the digestibility of its nutrients and rate of passage through the digestive tract. C. B. RODRIGUE and N. N. ALLEN, Univ. of Wisconsin, Madison.

The digestibility of long hay and of finely ground hay from the same lot was compared by the total collection method, with 8 cows. The cows were fed a milkfat-depressing ration of finely ground hay and grain until the milkfat depression was evident. They were then fed the trial ration of 2 parts ground hay and 1 part ground corn during a 10-day preliminary period, followed by a 10-day collection period. The ration was then changed to unground hay and corn, fed at the same rate through a 6-day adjustment period, followed by a 10-day collection period. Moisture, ash, crude protein, ether extract, crude fiber, and nitrogen-free extract were determined on feed and feces by A.O.A.C. procedures. In addition, lignin and cellulose were determined and total carbohydrates were calculated. Rate of passage was measured by using crystal violet dye on part of the hay at 1 feeding. The comparative average digestion coefficients, expressed as per cent for the combined hay-grain ration, were as follows, with the long hay value given first: Dry matter 65.9-61.4,** crude protein 68.0-68.0, ether extract 64.6-60.9,* crude fiber 46.3-30.8,** nitrogen-free extract 77.1-75.6, cellulose 52.6-39.4,** lignin 5.9-1.7,** total carbohydrates 74.6-67.6.** Other values were: Fat percentage of milk, 3.89-3.42;* 5% excretion time, in hours, 22.2-15.7;** 80% minus 5% excretion time, in hours, 56.9-44.0.*

* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)

P71. Effects of fiber content of the diet on milk production and hot weather discomfort in producing dairy cows. R. E. LEIGHTON and I. W. RUPEL, Texas Agr. Expt. Sta., College Station.

Twenty producing dairy cows (12 Holsteins and 8 Jerseys) were fed alternately for 4-wk. periods on diets equal in protein and net energy but markedly different in fiber. On the higher fiber diet, the cows consumed an average of 1.1 lb. of alfalfa hay and 2.4 lb. of sorghum silage per 100 lb. body wt. supplemented by a grain mixture containing 10.4% fiber. On the low fiber diet, the cows were limited to 0.75 lb. of alfalfa hay and 1.5 lb. of silage per 100 lb. body wt. and were fed a grain mixture containing only 4.1% fiber. During a mild weather trial there was no detectable difference in the milk production of the cows on the 2 diets, but during two 4-wk. periods in July and August highly significant differences were observed, not only in milk production but also in body temperature, respiration rate, and pulse rate. While on the low fiber diet the cows' average daily milk production was 2.16 lb. higher, their rectal temperatures were 0.31 degree lower, their respiration rates 6.77 per minute slower, and their pulse rates 4.46 per minute slower than when on the higher fiber diet. Milk production appeared to be more sensitive to the differences in diet and to changes in air temperature than were the other responses measured. There were significantly higher correlations between air temperature and either milk production or body temperature when the cows were on the higher fiber diet.

P72. Hay consumption of individual cows on limited grain feeding. J. E. LEGATES, W. R. MURLEY, and R. K. WAUGH, North Carolina State College, Raleigh.

Many workers have recognized the need for developing strains of dairy cattle that are capable of utilizing roughage to provide a higher proportion of their energy needs. Seventeen cows of the Ayrshire, Guernsey, Holstein, and Jersey breeds were fed U. S. No. 2 alfalfa hay free choice with added grain to provide 0.4 of maintenance requirements. Cows were observed during 2 successive lactations insofar as possible. Daily hay and grain consumption and milk production were obtained for each cow. Weight, heart girth, body length, and paunch circumference were also obtained.

The cows did not consume maximum amounts of hay early in lactation but reached a plateau after about 8 wk. on the trial. Consumption of hay from the 9th through the 12th week was used to characterize the individual cow's intake. Total hay intake/cow/day was most closely associated with body wt. and essentially independent of milk yield. The cows ranged from 2.65 to 4.37 lb. of hay consumed daily per 100 lb. of body wt. Breed differences in hay consumption per 100 lb. body wt. were significant, although the limited samples representing each breed are recognized.

P73. Variation in efficiency of feed utilization for maintenance of the dairy cow. J. W. THOMAS and L. A. MOORE, Dairy Husbandry Branch, USDA, Beltsville, Maryland.

An attempt has been made to estimate the extent of the variation in efficiency of the dairy cow. For this investigation the amounts of U. S. No. 1 alfalfa hay necessary to maintain dry open cows at constant body wt. was determined. The cows were stall-fed and exercised in dry lot for several hours each day. The amount of hay required to maintain wt. reflected the efficiency of the cow as well as the feeding value of the hay. Generally, the cows

repeated their rankings in efficiency when fed the different hays. More reproducible comparisons were observed when longer experimental periods were used. The average daily intakes on 4 different lots of hay were 13.2, 13.4, 12.2, and 11.6 lb. dry matter/cow/1,000 lb. live wt. The average daily wt. changes were +0.16, +0.26, +0.19, and −0.25 lb/cow, for each of the 4 lots of hay, respectively. The average length of the 4 trials was 84, 88, 68, and 140 days and the number of cows represented was 8, 12, 12, and 11, respectively. The average amount dry matter required to maintain a 1,000-lb. cow was about 12.5 lb., with a range of about 10.8 to 13.8 lb. when corrected for the small wt. changes observed. For 9 cows on 3 different hays the coefficients of variation were 4.5, 4.3, and 5.6%.

P74. Bone changes in molybdenum toxicity. H. F. ROBERTS, L. R. ARRINGTON, C. F. SIMPSON, and G. K. DAVIS, Florida Agr. Expt. Sta., Gainesville.

Skeletal abnormalities are characteristic of molybdenum toxicity in cattle and other species. The toxic syndrome with accompanying skeletal deformities was produced in rabbits with excess dietary molybdenum, and the changes were studied by chemical analysis and microscopic examination of the bone.

Crooked and bent front legs, which resulted in lameness, and a cartilaginous growth at the distal end of the radius were typical external symptoms. The bones from animals thus affected contained a normal percentage of total ash, calcium, and phosphorus but an increased amount of molybdenum. Microscopic examination revealed excessive cortical resorption in the subperiosteal region, thin periosteum, and thin epiphyseal lines, necrotic bone spicules, and eroded articular surfaces, which permitted escape of marrow. The marrow contained large vacuolated spaces with few hemopoietic cells, pink proteinaceous material, and hemolized red cells. Proliferating cartilage cells were detached from the epiphysis and irregularly arranged.

P75. Dried extracted coffee meal as a feed for dairy cattle.[1] R. E. MATHER and W. P. APGAR, JR., New Jersey Agr. Expt. Sta., Sussex.

Residue from soluble coffee manufacture was evaluated as a feed ingredient in 3 studies. Both dried extracted coffee meal and CoMol (69% liquid molasses equivalent dried with coffee meal) were studied. Sixteen Holstein cows in four 4 × 4 latin squares were used, 2 with coffee meal (0, 6, 12, and 18%) and 2 with CoMol (0, 8, 16, and 24%). Periods were 4 wk. or 3 wk. (one square). There was no significant effect on milk production, butterfat percentage, pulse rate, or flavor of the milk. Body wt. of cows on coffee meal was significantly reduced. Some cows refused coffee meal or CoMol at the highest levels but 2 other cows ate up to 5.2 lb. coffee meal for 100 days without refusal or undesirable effects.

Fifteen Holstein and Guernsey calves received starters containing 0, 10, or 20% CoMol from 5 to 25 wk. of age. Reduced rate of growth could be explained by lower consumption owing to poor palatability of CoMol.

Chromic oxide and chromogen techniques were used with 4 milking Holstein cows in 3 periods, giving estimates of 10.2 ± 3.0% protein digestibility, 1.3 ± 0.5% digestible protein, and 55.8 ± 3.6% TDN.

[1] Supported in part by Standard Brands, Inc.

P76. Grass-legume silage as the sole roughage for lactating cows. J. W. COBBLE and GLENN WILDES, Univ. of Rhode Island, Kingston.

This study was undertaken to evaluate grass-legume silage as the sole roughage for lactating cows. Twenty-four animals were used in 2 separate trials. Three 4-wk. feeding periods were employed in a double reversal design which compared an all-silage roughage ration with a silage-hay ration. Each group of 6 animals (silage, Group I; silage-hay, Group II) was fed a 17% protein grain mixture at the rate of 1 lb. of grain for each 3 lb. of FCM. Silage was fed to Group I at the level of 2 lb. of hay-equivalent per 100 lb. body wt. Group II was fed silage at the level of 1 lb. of hay equivalent and hay at a like amount per 100 lb. body wt.

Preliminary analysis of the data indicates higher production of FCM by the all-silage group with no significant differences in body wt. changes. Both groups on the average lost wt. slightly. Data will be available in respect to total dry matter intake and the relationship of TDN to FCM.

P77. Digestibility of grass and legume silages preserved with sodium metabisulfite and dried molasses beet pulp. I. R. JONES, J. V. BATEMAN, and J. R. SCHUBERT, Oregon State College, Corvallis.

Conventional digestion trials were run on forages preserved with *(a)* 200 lb. dried molasses beet pulp per ton and *(b)* 8 lb. sodium metabisulfite per ton. In 1953-54 a grass-legume mixture was ensiled; in 1954-55 first-cutting alfalfa containing approximately 10% grass was used. These silages were made in 12′ × 32′ wood-stave silos filled with alternate loads cut from the same fields by a field chopper.

Digestion coefficients were determined for each preservative-forage combination, the same animals being used for within-forage comparisons. Eight cows, including 3 sets of identical twins, were used to study each preservative. There were no significant differences among

the 4 silages in digestion coefficients for dry matter, crude protein, ether extract, crude fiber, and NFE.

On a dry basis, the grass-legume silage averaged 67.42% and 67.12% calculated TDN when preserved with beet pulp and metabisulfite, respectively. For alfalfa silage, the values were 67.47% TDN for beet pulp and 65.33% TDN for metabisulfite.

On an as-fed basis, silage made with dried molasses beet pulp averaged 30.19% dry matter, and that preserved with sodium metabisulfite averaged 25.81%.

P78. Effect of various preservatives on seepage from high moisture silage. P. M. REAVES and R. E. BRUBAKER, Virginia Polytechnic Institute, Blacksburg.

Chopped alfalfa forage containing more than 80% moisture was ensiled in miniature silos (metal barrels). These silos held approximately 250 lb. of chopped forage. Weights were added to give about 275 lb. of pressure per sq. ft. This would be equivalent to a depth of 8 ft. of settled silage. The following treatments were used: none, molasses, corn and cob meal, brewers' dried grains, beet pulp, sulfur dioxide, and wilting. Duplicate silos were used for each treatment.

Results were secured for a period of 30 days for one half of the silos and for a period of 67 days for the other. The percentages of moisture content of the silages after 67 days were (in order of treatments listed above): 79.2, 77.3, 76.1, 74.9, 75.5, 78.2, and 62.5. The amount of seepage in lb. and the percentages of solids in the seepage were (in the same order): 12.7, 7.6; 16.4, 10.0; 0.7, 11.9; 0.4, 14.1; 0.0, 0.0; 32.6, 7.7; 0.0, 0.0. The pH and the acidity calculated as lactic acid were also determined.

P79. Effects of sodium metabisulfite and iodized sodium chloride on preservation and milk production value of alfalfa silage with levels of protein supplementation. H. H. VOELKER and E. R. BECKER, South Dakota State College, Brookings.

Wilted alfalfa (68-70% moisture) was preserved in bunker and in upright silos. Half of 1 bunker was treated with sodium metabisulfite (10 lb/ton). Half of a second bunker was treated with 1% iodized salt. In 3 trials, 48 cows (Brown Swiss, Guernseys, and Holsteins, 8 per group, cross-over design, 12- and 16-wk. trials) were individually fed. Half of each group received concentrate supplementation (16.1, 14.4, and 13.2% protein) compared to corn-oats combinations (10.5 and 11.5% protein). One lb. of concentrate was fed per 3 lb. FCM. Six lb. of alfalfa hay, 14.4% protein, were fed daily per cow. Daily ad libitum consumptions of silage were: control, 51.9 lb.; salt-treated, 52.6 lb.; bisulfite-treated, 49.6 lb. Av. wt. losses of alfalfa silage–fed cows were: 21 lb., low-protein group; 48 lb., high-protein groups. These losses were recovered in 30 days' post-experiment feeding of corn silage ad libitum plus alfalfa hay. Daily production (FCM) averaged 29.4 lb. for the bisulfite group; 29.9 lb. for the no-bisulfite group. There were no significant differences in production due to level of protein in concentrates. The only significant difference in silages found upon complete chemical analyses was a carotene content approximately 4 times as great in bisulfite-preserved silage as in controls. The pH levels of silages were: bisulfite, 4.85; control, 5.14; salt-treated, 5.4.

P80. A comparison of chopped and unchopped silage stored in bunker silos. C. H. GORDON, H. G. WISEMAN, C. G. MELIN, Dairy Husbandry Research Branch, and R. J. McCALMONT and L. E. CAMPBELL, Agricultural Engineering Research Branch, USDA, Beltsville, Md.

Second cutting grass-clover forage mixture was stored without wilting in 2 bunker silos (15′ × 66′ × 8′). The crop yield was light and dry (29% dry matter) because of dry weather. One bunker was filled with unchopped forage, the other with chopped forage. Both forages were continually tractor-packed during filling and for 1 day following, then covered with polyvinyl chloride (54″ × 0.008″) seamed with 4-inch tape and weighted at the edges. Composition changes, spoilage, nutrient preservation, and feeding value for dairy cows were determined for both silages after a storage period of 6 mo.

Harvesting, silo filling, and silage removal of the unchopped material were more tedious. Total losses of stored dry matter were 28% and 31% for the unchopped and chopped silages, respectively. Small air leaks appeared to have destroyed the usefulness of the plastic cover for preventing air-induced spoilage. Proximate analyses of the 2 silages as stored and after storage were very similar. More trapped air in the unchopped silage was associated with higher pH, butyric acid, and ammoniacal nitrogen values and lower lactic acid values. Cows consumed more silage dry matter, gained more live weight, and produced significantly more milk when fed the chopped silage.

P81. Effect of grain on the digestibility of grass silage. E. A. KANE and W. C. JACOBSON, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

The digestibility of 3 differently treated silages was determined in a total collection digestion trial in which grain was fed in varying amounts according to milk production. No significant difference for silages or cows in dry matter digestibility or palatability (amount of dry matter consumed) was observed. The digestibility F-value for cows approached significance (5%).

When the crude fiber data were tested similarly, cow differences proved to be highly significant (1%) for both crude fiber digestibility and amounts of crude fiber consumed. Covariance analysis revealed that when the amounts of grain fed were equalized, no significant difference for cows was obtained. The highly significant differences for cows reported above were due, apparently, to varying amounts of grain fed.

Results of this experiment indicate that added carbohydrate failed to affect significantly the dry matter digestibility of the silage but did affect the crude fiber digestibility of silage in a highly significant manner. This indicates that the crude fiber component of the ration, under the conditions of this experiment, was more sensitive than dry matter for the purpose of studying relationships between changing carbohydrate intakes and utilization of forage nutrients.

P82. Ear-Corn silage as grain for dairy cattle. A. D. PRATT and C. F. ROGERS, Ohio Agr. Expt. Sta., Wooster.

Twenty-four tons of ear-corn silage were fed as a substitute for mixed grain rations to 4 groups of heifers and to 2 groups of milking cows. When the roughage supplied adequate protein, ear-corn silage replaced the entire grain ration; when protein would otherwise be inadequate, protein concentrate supplemented ear-corn silage.

The ear-corn silage contained 54% dry matter, 4.6 γ carotene per gram on a dry basis and 9.4% protein also on the dry basis. The silage weighed an average of 56 lb. per cu. ft. and contained an estimated 24 lb. of digestible nutrients per cu. ft.

With both heifers and milking cows, replacement of dry grain mix by moist ear-corn silage on a dry matter basis (exclusive of cob) resulted in equally satisfactory physical condition, wt. gains, and milk production.

P83. Development of organic acids in silage. H. M. IRVIN, C. W. LANGSTON, and C. H. GORDON, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Organic acids occurring in 3 cuttings of orchard grass and of alfalfa silages during the first 40-60 days of the ensiling process were determined by the chromatographic technique developed by Wiseman. Five 4′ × 8′ steel silos were used for storing the crops. The treatments for each of the 5 silos were varied in an attempt to produce gradations of good to poor quality silage. The acids studied in this work were generally butyric, propionic, acetic, formic, lactic, and succinic.

The acid content of the fresh grass or alfalfa placed in the silos was usually under 1% on a dry matter basis with acetic acid present in the highest percentage. In silages with a mediocre or poor quality, butyric acid was present after 5-8 days. Under similar conditions lactic acid increased during the first 5 days and then decreased. Acetic acid seemed to fluctuate, often paralleling the behavior of butyric acid. In silages of good quality, acetic acid increased the first 2 days after ensiling and showed a slow increase for 40-60 days. Lactic acid increased markedly the first 8-12 days to as much as 8-10% with no detectable butyric acid present. In all silages, propionic, formic, and succinic acids occurred in relatively small amounts.

P84. Chromatographic separation of silage acids. H. G. WISEMAN and H. M. IRVIN, Dairy Husbandry Research Branch, USDA, Beltsville, Maryland.

A celite column with alphamine Red-R as internal indicator has been developed to separate silage acids ranging from butyric to succinic. The method is designed to eliminate steam distillation, or ether extraction of acids, and mechanical collections with numerous titrations. Silage samples are soaked for 1 wk. with an equal volume of 0.6 *N* H_2SO_4, and the expressed liquor is centrifuged to provide extracts for analysis. These aqueous solutions of acids, 1-2 ml., are added to dry cap material on the column and homogenized, and the acids are developed as even zones by acetone and Skellysolve B mixtures. Recoveries of known amounts of butyric, propionic, acetic, formic, lactic, and succinic acids were 99.2, 101.0, 99.7, 99.6, 97.5, and 98.6%, respectively.

P85. In vitro studies on methanogenic rumen bacteria. W. O. NELSON, R. A. OPPERMANN, and R. E. BROWN, University of Illinois, Urbana.

Theoretical yields of methane and carbon dioxide were obtained from stabilized enrichment cultures of mixed rumen bacteria adapted to formic and/or acetic acid. Attempts to stabilize cultures on butyric or propionic acid at 37° C. were unsuccessful. However, cultures that produced methane from butyric acid were obtained at 45° C. An incubation period of approximately 3 wk. was required before substrate utilization, gas volumes, and the ratio of carbon dioxide to methane approached theoretical values. Thereafter, constant activity was maintained for 4 mo. or longer by daily addition of substrate. Major changes in flora occurred in the first 7 to 10 days of incubation. A gram-negative micrococcus predominated in all stabilized cultures. Associated organisms included a gram-positive macrococcus and a gram-variable long, slender, nonsporulating rod. The latter form was more in evidence when formic acid was fed. Changes in relative concentrations of the above types occurred when stabilized cultures were fed a foreign substrate. The acetic acid adapted culture differed from the formic acid cultures in sensitivity to antibiotics, dyes, and other inhibitors.

P86. Mechanism of methane formation by bovine rumen bacteria and the effect of hydrogen acceptors and donors on methane production. J. J. McNeill and R. N. Doetsch, Univ. of Maryland, College Park.

An in vitro study of various aspects of the methane and hydrogen metabolism of bovine rumen bacteria has been conducted by employing bacterial cell suspensions recovered from rumen contents by differential centrifugation. Methane formation was demonstrated with carbon dioxide and molecular hydrogen as the only substrates, indicating that the carbon dioxide reduction mechanism of methane formation is operative in the rumen. The presence of inorganic and organic hydrogen acceptors (nitrate, sulfate, riboflavin, etc.) decreased methane production from various substrates primarily by competition with carbon dioxide for active hydrogen. Likewise, a hydrogen donor, molecular hydrogen, increases methane production by supplying more hydrogen for the reduction of carbon dioxide. The significance of these observations in relation to in vivo methane formation will be discussed.

P87. The effect of D-limonene on in vitro dissimilation of carbohydrates and methane formation by rumen bacteria. R. E. Brown, Anatole Crane, and W. O. Nelson, Univ. of Illinois, Urbana.

McNary *et al.* (*Food Technol.*, **5**: 319. 1952) reported that methanogenic bacteria in citrus waste treatment systems were inhibited by the presence of minute concentrations of the essential oil of citrus peel. The data suggested that carbohydrate dissimilation was blocked at the fatty acid stage by D-limonene. Since analogous inhibition of methane formation in the rumen may have application in ruminant physiology, the effects of D-limonene on carbohydrate dissimilation and methane formation by rumen bacteria were investigated. Accordingly, a series of experiments was conducted to measure the effect of varied levels of D-limonene on the activity of stabilized enrichment cultures of methanogenic rumen bacteria adapted to formic and/or acetic acid. A second series of experiments was conducted to measure the effect of D-limonene on carbohydrate dissimilation by rumen bacteria. High levels of limonene inhibited cellulose digestion but did not stop starch and cellobiose degradation. Levels of limonene which permitted cellulose digestion inhibited methane production by stabilized enrichment cultures adapted to formic and/or acetic acid. The addition of D-limonene to rumen fluid supplemented with cornstarch resulted in the accumulation of formic and lactic acids.

P88. Nutritional requirements of rumen bacteria. J. J. Gilroy and R. N. Doetsch, Univ. of Maryland, College Park.

The ability of certain unidentified factors to stimulate the activity of rumen microorganisms in vitro has been investigated. Commercial rumen products, protein digests, and nutritionally rich culture media were compared with rumen fluid for their ability to support growth of a mixed population of rumen microorganisms. Pure cultures were obtained from rumen fluid in high dilutions. All isolations were made from rumen fluid agar. The ability of nutritionally rich media to support growth of these organisms was determined. Reinforced clostridial medium (Hirsch and Grinsted) and Eugonagar (BBL) were employed with slight modifications. Reinforced clostridial medium supported the growth of all isolates tested at a rate comparable to that of rumen fluid medium. Visible growth on Eugonagar of approximately one-third of the isolates was observed only after incubation periods of 4 to 10 days. All isolates eventually grew on this medium.

P89. A time study of the relative concentrations of fatty acids occurring in the rumen of cattle on different rations. T. N. Wegner, G. M. Ward, and L. E. Washburn, Colorado A. & M. College, Ft. Collins.

Two dry dairy cows were used for this experiment. Both were fitted with a plastic, screw-cap, rumen fistula plug. The experimental rations were fed once a day at 10 A.M. Two weeks were allowed for the cattle to adjust to each new ration. Feed was restricted for 24 hr. prior to the day of collection. A 0-hr. sample was used as the base concentration of fatty acid. This sample was taken 5 min. prior to the normal time of feeding, followed by samples at 2, 4, and 6 hr. after feeding. The samples were taken from the middle of the ventral sac of the rumen. Two collections were made on each ration, with a 7-day interval between collections. Acetic, propionic, and butyric acid fractions were determined by direct chromatography of the rumen fluid on packed silica gel columns. The experimental rations were: (1) alfalfa hay, corn silage, and grain; (2) all alfalfa hay; (3) all alfalfa silage; and (4) pasture. Analyses to date show the concentrations of acetic, propionic, and butyric acid (m*M*/100 ml. rumen fluid) to be, respectively: Ration 1, 0 hr.: 14.97, 4.83, 4.48; 2 hr.: 17.57, 5.62, 6.76; 4 hr.: 7.88, 1.72, 2.64; 6 hr.: 6.47, 1.66, 2.01. Ration 2, 0 hr.: 5.52, 1.20, 1.00; 2 hr.: 4.67, 1.11, 1.11. Further analyses will be presented.

P90. Blood and rumen substances of cows on different feeding regimes prepartum and postpartum. T. R. Lewis and D. R. Jacobson, Univ. of Maryland, College Park.

Twenty cows were divided into 2 equal groups and placed on experiment at least 6 wk. prior to parturition. Group I cows were fed alfalfa hay, free choice, prior to parturition and alfalfa

hay, free choice, plus the regular herd mix, after parturition. After parturition, the Group I cows were brought to a high level of grain feeding as rapidly as possible. Group II cows were fed alfalfa hay, free choice, plus the regular herd mix both prepartum and postpartum. The grain for Group II was fed at a level of 8 lb./1,000 lb. body wt. prepartum and was increased whenever necessary postpartum. Two rumen samples were drawn from each cow prepartum and twice weekly for the first 2 wk. postpartum, and once weekly thereafter. Blood samples for glucose and ketone body determinations were drawn at the same time. The experimental period extended to 26 days postpartum. Both the blood glucose levels and the proportions of the rumen volatile fatty acids were similar for the 2 groups. Total rumen volatile fatty acid concentrations were slightly higher for Group II. No marked changes were observed in the proportion of the rumen volatile acids when the cows were changed from an all-hay ration to hay plus concentrate. The cows that were fed high levels of grain prepartum consumed larger amounts of grain immediately postpartum.

P91. Absorption and dissimilation of lactates added to the bovine rumen and the resulting effects on blood glucose. F. G. HUETER, Univ. of Maryland, College Park.

Two permanently fistulated cows were fed on a constant diet with controlled water intake. Various forms of lactic acid were administered via the rumen fistula. The effects of the added substrates on rumen lactic acid, volatile fatty acids, blood lactic acid, and blood glucose were then determined. These studies indicate that degradation of lactates by bovine rumen microorganisms results in increases in rumen VFA, mainly propionic and butyric acids. Both calcium lactate and sodium lactate caused appreciable increases in rumen propionic acid concentrations, whereas only sodium lactate caused marked increases in blood lactic acid and blood glucose, indicating that the increases in blood glucose were due primarily to absorption of lactic acid from the more soluble lactate and not to absorption of propionic acid. An in vitro dissimilation of sodium lactate by mixed suspensions of bovine rumen bacteria appeared to be indicative of in vivo rumen occurrences. In studies with cows with ketosis, which were resistant to the usual therapeutic dosages of glucocorticoids and glucose precursors, it was observed that sodium lactate per os was much more effective than calcium lactate. Three lb. of sodium lactate per day for 2 days resulted in a remarkable decrease in urine ketones to normal within 48 hr. but produced diarrhea. Three lb. per day of a combination of calcium lactate and sodium lactate (2:1) was administered without adverse effects, and this combination proved to be a much more effective treatment than calcium lactate alone.

P92. Changes in rumen and urine nitrogen and volatile fatty acids in relation to time after feeding alfalfa or mixed hay. H. R. CONRAD and J. W. HIBBS, Ohio Agr. Expt. Sta., Wooster.

Ammonia and urea nitrogen in hourly urine collections from a Foley type indwelling catheter along with rumen levels of ammonia nitrogen were used to indicate absorption from the rumen after feeding either alfalfa or mixed hay. Ammonia nitrogen in the rumen juice increased sharply during the first ½-hr. after feeding alfalfa hay, reaching a maximum in 1½ hr., and returned to normal within 4 hr. In contrast, no changes were noted when mixed hay was fed. Urea and ammonia nitrogen excreted in the urine increased sharply also during the first 1½ hr. after alfalfa was fed and then dropped sharply to the prefeeding level after 2½ hr. However, a more marked and sustained rise in urine nitrogen excretion occurred during the 3- to 9-hr. period after feeding, a time when rumen ammonia levels were at a minimum. No changes were observed in urine nitrogen levels when mixed hay was fed. These results indicate that when alfalfa hay was fed, approximately 2% of daily nitrogen intake was lost through the rumen wall. Acetic and butyric acid in the urine increased abruptly after feeding either alfalfa or mixed hay. These increases paralleled increases in the rumen but were relatively steeper. Only traces of propionic acid were found in the urine.

P93. Effect of a detergent on incidence and severity of bloat and on characteristics of rumen fluid of cattle. J. T. BLAKE, R. S. ALLEN, and N. L. JACOBSON, Iowa State College, Ames.

An alkyl aryl sodium sulfonate type detergent was administered (daily via capsule) alternately to 2 groups of cattle (11 animals in each group) grazing alfalfa. Although lower levels had little effect, a significant reduction in bloat incidence and severity occurred at a daily rate of 3 g. of detergent per 100 lb. body wt. Grazing habits apparently were unaltered by treatment. Foaminess, foam half-life and "ingesta-volume-increase" of rumen fluid were markedly increased; surface tension was moderately increased; and viscosity was slightly increased during bloat (as compared to contemporary nonbloated animals and to values for the same animals when not bloated). All of these measurements were decreased by detergent administration. Specific gravity and pH were slightly lower during bloat and higher during treatment.

Bloat was produced by oral administration of fresh alfalfa juice. Addition of detergent to the juice had no apparent effect on development of bloat and altered some rumen fluid properties differently than in animals on pasture. Foam half-life increased and surface

tension decreased, but effects on other properties were variable.

The detergent had little effect in relieving chronic bloat. Trisodium phosphate, given for pasture bloat, and cholesterol, for pasture and alfalfa-juice bloat, were ineffective as prophylactics.

P94. Factors affecting the dry matter digestion of various roughages in the artificial rumen. B. R. BAUMGARDT and D. L. HILL, Purdue Univ., Lafayette, Ind.

The purpose of this study was to investigate the dry matter digestion of various forages in the artificial rumen and to study sources of error or bias that might occur. The procedure described by Huhtanen *et al.* (*J. Dairy Sci.*, 37: 328. 1954) was modified by using sacs of known weight and a washed cell suspension of rumen fluid. Some of the important factors studied included: length of incubation period, buffer nutrient solution used, ratio of substrate to inoculum, collection and preparation of inoculum, type of roughage, and losses occurring when only water was used as "inoculum." Some of the typical dry matter losses obtained were as follows: Ladino clover, 65%; alfalfa hay (bud stage), 58%; Ky. bluegrass (mature), 40%; and wheat straw, 30%. The within-trial coefficient of variation was usually less than 2%. Fourteen forages were incubated with only a water inoculum. Samples of these same forages were incubated with a washed cell inoculum. There was a significant correlation between the per cent dry matter losses obtained by these 2 methods ($r = 0.927$).

P95. An inexpensive urine-collecting apparatus for dairy cows. P. A. PUTNAM, R. G. WARNER, and J. K. LOOSLI, Cornell Univ., Ithaca, N. Y.

The apparatus to be described is inexpensive, is easy to construct, and consists of materials which are readily available. The materials are: coat-hanger wire, 3/16-inch rubber sheeting, foam rubber, rubber tubing, rubber cement, branding cement, a tire-patching kit, friction tape, copper wire, aluminum sheet metal, 3-inch Gooch tubing, an old inner tube, bolt or metal rod, two kneeling pads, two small pieces of plywood, and a fiber strap. The apparatus may be completely assembled in an afternoon and is ready for use within 12 hr. The cost is about $10.

This apparatus allows for an uncontaminated collection of urine and/or feces of stanchioned cows. There has been little or no irritation to the cow after periods of as long as 11 days. The elasticity of the harness is believed to be a major factor in eliminating irritation while facilitating a firm fit. The apparatus has the additional advantage of being light and flexible, thus placing a minimum of stress on the animal. It also contacts only the external and observable surfaces of the animal.

Apparatuses constructed from these materials have been used for 20 digestibility trials on 6 different cows. The uniformity of experimental results to date illustrates its effectiveness.

EXTENSION SECTION

E1. 4-H dairy calf placement programs. R. A. PORTERFIELD, The Ohio State Univ., Columbus.

In each of the 48 states, 4-H dairy club work ranks as a major project. The primary objective is to teach boys and girls improved methods of dairying and to provide them with an opportunity to learn by doing. To carry out this objective it is important that club members obtain acceptable animals for their first projects. Selection and placement methods in the United States, Hawaii, Alaska, and Puerto Rico have been determined by means of a survey. Results of this study indicate that 50% or more of the 4-H club members obtain animals for project work from the home herd. To date, the incomplete results show that approximately 1,200 animals are purchased through special sales for 4-H and FFA members, nearly 1,100 animals are secured through heifer chains, and about 250 animals are purchased at "Heifer Parties." Other project animals are purchased through routine consignment sales and at private treaty. All states encourage 4-H dairy club members to purchase heifers from dams with known production records. The most frequent suggestion is that the dams have records of 400 lb. or more of butterfat.

The survey indicates that county agents, associate county agents, local 4-H club leaders, and purebred breeders are key personnel in the selection of nearly all dairy project animals.

E2. A look at our Junior Dairy Show program—A panel. C. W. NIBLER, Lincoln, Nebraska; FRED GAUNT, Milltown, New Jersey; H. L. MANN, University Park, Pennsylvania; W. N. PATTERSON, Blacksburg, Virginia; and H. A. WILLMAN, Cornell Univ., Ithaca, N. Y.

In recent years 4-H dairy enrollments and the size of Junior Shows have grown rapidly. Along with this trend, many states have tried to make the exhibition of cattle more educational to boys and girls and more valuable to the industry from a herd-building standpoint. One improvement in the conduct of Junior Shows has been a more general use of the group, or Danish, system of judging. In one state, a survey of exhibitors, parents, and agents showed an 83% preference for the group system as contrasted to the 1, 2, 3, 4 consecutive order plan. In another state 80% of the county fairs use a combination of the 2 systems. This latter plan seems to be especially valuable in states where competition is carried beyond the county and district level.

E3. Worm-parasite control in dairy management. A. C. TODD, Univ. of Wisconsin, Madison.

Infectious disease is widely considered catastrophic and beyond prevention; its control is considered not to be a part of dairy management but to belong to a field separate from production. Dairy veterinary treatment today utilizes techniques that can prevent infectious disease almost entirely, and an efficient dairyman can now determine his own infectious disease losses. The future development of dairy production should include a greater knowledge of the kinds of disease involved and basic disease prevention measures.

One misconception commonly shared by dairymen and veterinarians is that the importance of infectious disease is derived entirely from mortality rates. In the light of modern intensive production, the important diseases are those that result in universal morbidity, and these are the diseases that traditionally escape attention. No cattle infections are more omnipresent in American dairy herds than those caused by internal parasites. These infections damage cattle without ordinarily being brought to the dairyman's attention; in fact, American standards for "healthy" animals are based on parasitized animals. The importance of subclinical parasitism is shown by improvement in the animals' appearance and production following both curative and preventive treatment. A simple, inexpensive system of preventive treatment, which controls 65-70% of all internal parasitism, will be illustrated. The report is based on field work with Wisconsin herds.

E4. Putting flavor into the quality program. Alec Bradfield, Univ. of Vermont, Burlington.

During the past 50 years numerous attempts have been made to write a criterion of milk quality. Safety, cleanliness, keeping quality, low bacterial content, appearance, and nutritive value are all mentioned, but nowhere has there been any attempt to put flavor into such a standard.

A great deal of research has been done on undesirable flavors in milk. The basic causes of rancid, feed, and many other off-flavors have been discovered. However, farmers are still permitted to feed silage before milking, to put the milk from late lactation and stripper cows onto the market, and to engage in other practices that render milk unpalatable.

Various reports have corroborated studies in Vermont which show that nearly 70% of market milk has some undesirable flavor. Under the program that has been conducted in Vermont during the past year the incidence of undesirable flavors has been reduced to less than 35%. Flavor should be made an integral part of the quality program; it should be given at least equal importance with all the other quality tests.

E5. Making dairymen milk-flavor conscious. W. A. Dodge, Univ. of Vermont, Burlington.

Dairymen in Vermont, as well as other states, are spending large sums of money to advertise milk. Supplying dairymen with information on the causes of off-flavors in milk is the problem. It was solved this way in Vermont:

1. Telling dairymen just how their milk does taste.
2. Convincing them of the presence of off-flavors by inviting them to join in the tasting.
3. Informing them as to how they can correct off-flavors.

Teams of tasters were trained for every milk-receiving station. These teams consisted of the milk inspector covering the plant, a plant employee or fieldman, and a dairyman shipping to the plant. Half-pint samples of each producer's milk were scored as "good," "fair," or "poor." A score sheet showing the rating of his milk and an indication of the nature of any off-flavor was delivered to each producer, together with a copy of Vermont Extension Brieflet No. 956, which tells how to correct off-flavors. Dairymen either become interested and correct off-flavors or find themselves looking for a market.

This all means that a drink of Vermont milk tastes like "more."

E6. Some environmental adjustments which aid in sire evaluation. H. W. Carter, Cornell Univ., Ithaca, N. Y.

The production records of over 40,000 artificially sired cows have shown that for accurate sire evaluation adjustments should be made for the following: (1) year of freshening, (2) season of freshening, and (3) level of herds in which the records are made. By dividing the year into 6-mo. periods most of the variation due to month of freshening can be accounted for. These periods are March 1 through Aug. 31 and Sept. 1 through Feb. 28.

By comparing each record of an AI sired cow with the production of the other cows in the same herd freshening during the same 6 mo., adjustments were made for both year and season of freshening. This adjustment showed that the average level of herds where sires' daughters made their records varied by as much as 40 lb. of butterfat. C. R. Henderson and associates found that 60% of these differences between herds was reflected in the production of a sire's daughters.

These simple adjustments have decreased the regression for number of daughters of AI sires from $\frac{N}{N+34}$ to $\frac{N}{N+12}$. N represents the number of tested daughters of a sire. This regression is used for predicting the production of future daughters of an AI sire based on the production of the present number of tested daughters.

E7. Demonstration of some management influences on lactation yields (D.H.I.A. 718's). MARY M. GOODWIN[1] and R. E. ERB, State College of Washington, Pullman.

Herd averages as reported by D.H.I.A. testers for 1954 were compared to lactation averages as calculated in compiling Herd Management Evaluations. Only normal lactations with 1953 freshening dates were used. Herds (713) with lactation records reported averaged 655 lb. more FCM per cow than herds (237) with none reported. Average length of record more nearly approached 305 days as the herd average increased from less than 300 to over 500 lb. of fat per cow. During 1953 and 1954, 8,643 previous dry periods were reported. The method of fitting constants was used to delete effects of breed and D.H.I.A. Logs of age were used to compute age regression. Age regression materially decreased the variance of mature equivalent milk records but did not decrease the variance of mature equivalent fat records. The average for all records was 9,713 lb. of milk and 431 lb. of fat. On an M.E. basis, these averages were 10,212 lb. of milk and 453 lb. of fat. When actual records were adjusted for breed, association, and regression on age, those with 0 to 9 days-dry before a record averaged 1,459 lb. less of milk and 62 lb. less of fat than records with 50 to 59 previous days-dry. These differences are essentially the same as those found earlier on a smaller sample.

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E8. Costs and returns from pasture on Florida dairy farms. C. W. REAVES, Univ. of Florida, Gainesville.

The study concerned home-grown feed nutrients as pasture and roughages obtained by Florida dairy herds and pasture costs compared with returns. It covered 44 commercial herds and 57 herds in D.H.I.A. By inverse calculation, nutrients from home-grown forages were found to provide about 27% of the total feed for commercial herds. Herds in D.H.I.A. secured 34% of total requirements from the farms. Production was higher and cost per 100 lb. milk lower for herds obtaining 30 to 50% of TDN from pasture than for groups with less pasture.

Pasture on 19 farms cost $39.66 and returned $83.22 per acre annually, based on cost of TDN in citrus pulp at $50.00 per ton. TDN from pasture cost $1.47 per 100 lb.

E9. The place of herd management in mastitis control. J. F. CONE, Pennsylvania State Univ., University Park.

Management practices concerned with mastitis control include both heredity and environment. In some cow families the udders tend to break away early and become pendulous; malformations that lead to injury have also been noted, and undoubtedly other factors are involved in the hereditary tendency to mastitis.

Abuses in milking are important factors. Improper preparation for let-down, irregularities in timing the milking operations, and improper operation and care of the machine may contribute to the development of mastitis. Leaving the machine on the cow too long is particularly objectionable. Crowding the cows in the stables (stalls too short or too narrow), insufficient bedding to prevent bruising and chilling of the udder, and poor ventilation lead to udder trouble. These factors apply not only during the normal lactation period but also during the dry stage and the prepartum and postpartum periods. Malnutrition may lead to susceptibility to infection. Forced feeding for high production also may cause mastitis, particularly in some families. Sudden changes in diet should be avoided. Drying off should be preceded by a lower level of feeding to decrease production.

A high percentage of cases of mastitis can be avoided by proper herd management. Therapy without correction of the environmental cause is ineffective.

E10. Diagnosis and treatment of mastitis. W. N. PLASTRIDGE and H. H. HALE, Univ. of Connecticut, Storrs.

Diagnostic procedures selected after extensive tests are (1) incubation of milk samples for 14 to 18 hr. at 37° C., (2) microscopic examination to determine the leucocyte count and presence of streptococci, (3) culturing of streptococcus-free samples with leucocyte counts in excess of 1,000,000 per ml. on blood agar, and (4) culturing of samples containing streptococci on a selective medium to differentiate *Streptococcus agalactiae* from other streptococci. Tests found inadequate for the detection of *S. agalactiae* are: catalase, bromthymol-blue, Hotis, sediment content, strip-cup, and Whiteside.

The following program combined with sanitary measures to reduce spread of infection has been found effective in eliminating *S. agalactiae:* (1) a quarter sample test on all milking animals, (2) immediate treatment of all infected quarters, (3) a retest of all cows 14 to 21 days after treatment, and (4) repeated tests followed by treatment at intervals of 3 to 4 wk. until *S. agalactiae* is eliminated. Severe acute systemic attacks of mastitis are not caused by *S. agalactiae;* they are usually sporadic in nature and require immediate intravenous or intramuscular therapy.

Of 1,151 herds in the Conn. control program, 666 were free of *S. agalactiae* on the last test made in 1955. Production records on D.H.I.A. herds show that the average production increased by about 10% in 91 herds in which *S. agalactiae* was eliminated and decreased about 10% in 24 herds that were *S. agalactiae*-negative and that later became infected.

E11. A state mastitis control program. H. G. HODGES, N.Y.S. Veterinary College, Cornell Univ., Ithaca, N. Y.

The New York State Mastitis Control Program was established in 1946 with an appropriation of $75,000. Today annual appropriations amount to $160,000. The program is an activity of the Medicine Dept. of the Veterinary College at Cornell Univ.

There are a basic research laboratory and a field laboratory at Ithaca and 5 other field laboratories located throughout the state. Each laboratory is staffed by a veterinarian and laboratory technicians. The field veterinarian is a specialist on bovine mastitis. He works with veterinarians, dairy farmers, extension service people, and many others. Herd examinations are made by him in cooperation with a veterinary practitioner or by the practitioner alone.

Herd surveys consist of a clinical examination of the udder and its secretions and the collection of strict foremilk samples from each quarter for laboratory analysis. In addition, a careful evaluation of the environment of the cow is made. This is accompanied by a program of treatment by, or under the close supervision of, the veterinary practitioner. Correction of faulty management practices is also made. Without the combination of adequate diagnosis, good environment, and proper treatment, little can be done to control the disease.

The recognition of abnormalities in milk secretions in New York State has done much to create a favorable reaction, for dairymen can readily see improvement in milk secretions. This phase of the program is regarded favorably by many milk quality agencies, both industrial and municipal.

MODIFICATION OF THE REFRACTIVE INDEX METHOD FOR THE DETECTION OF FOREIGN FATS IN DAIRY PRODUCTS[1]

V. R. BHALERAO AND F. A. KUMMEROW
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It has been pointed out previously *(3)* that differences in the mixed fatty acid composition *(6, 8, 9, 10)* and in the unsaponifiable content *(2, 4)* have served as a means of identification of foreign fats in butterfat. It is, however, possible to prepare from tributyrin coconut oil and beef tallow a synthetic fat mixture *(3)* with almost the same fatty acid composition as butterfat. The methods based on tocopherol and sterol content will detect only vegetable fats. Moreover, the processors of vegetable and animal fats have developed new techniques for modifying fats *(11)*, the physical and chemical properties of which may be similar to those of butterfat. Since these modified fats are relatively new, very little information is available about them. There is, therefore, no sound basis for the identification of these fats.

Recently it has been observed that when butterfat was fractionated from absolute ethyl alcohol at 20° C., the substitute fat was concentrated in the alcohol-soluble or insoluble fraction *(3)*. Subsequently, it was possible to detect its presence by following the refractive indices of these fractions. Difficulty, however, was sometimes encountered when a mixture of coconut oil and hydrogenated fat was used as an adulterant. Further refinements were therefore carried out in the refractive index method in order to develop a method based on the differences between glyceride structure of butterfat and other edible fats. The method described in this paper can be used for the detection of all possible combinations of foreign fats in admixture with butterfat.

EXPERIMENTAL PROCEDURE

Fractionation of butterfat from absolute alcohol. Fifty g. of butterfat was weighed out into a 1-l. Erlenmeyer flask, 500 ml. of hot absolute ethyl alcohol was added to the flask, and the mixture was shaken thoroughly until a clear solution was obtained. The flask was cooled to 20° C. and maintained at this temperature for 2 hours. The mixture was filtered and the precipitate was washed once with about 50 ml. of absolute ethanol. The filtrate and the washing were combined and freed of solvent on a steam bath and finally dried under vacuum. The refractive index of the soluble fraction was determined at 40° C. The precipitate was dried on a steam bath and transferred to a vacuum oven for final drying, and the refractive index of the melted fat was determined at 40° C.

Fractionation of alcohol insoluble fraction from acetone. Ten g. of the alcohol-insoluble fraction was dissolved in 100 ml. of acetone, the solution was cooled to

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0° C., and the flask was kept at this temperature over night. The solution was then filtered at 0° C. and the precipitate was washed once with 10 ml. of acetone, which had been previously cooled to 0° C. The filtrate and the washing were combined and freed from acetone, first on a steam bath and finally under vacuum. The precipitate of the acetone-insoluble fraction was freed of acetone in the same way. In all, three fractions were obtained: *(a)* alcohol-soluble fraction, *(b)* alcohol-insoluble, acetone-soluble fraction, and *(c)* alcohol-insoluble, acetone-insoluble fraction.

Estimation of trisaturated glyceride. The trisaturated glyceride content of the alcohol-soluble fraction was determined by the following method. Five g. of the fat was dissolved in 200 ml. of acetone, and 12 ml. of glacial acetic acid was added. The solution was heated to boiling, and finely powdered potassium permanganate was added in small amounts with vigorous shaking after each addition, then 6 ml. of acetic acid was added for every 16 g. of potassium permanganate used for the oxidation. The concentration of acetic acid was thus maintained between 3 and 6%. Usually 8 to 10 g. of potassium permanganate was required for every gram of fat for complete oxidation. After all the permanganate was added, the contents of the flask were gently refluxed for about 12 hours on a steam bath.

At the end of the oxidation period, the acetone was removed by distillation the last traces being eliminated under vacuum. About 150 ml. of water was added and the mixture was heated on a steam bath with agitation until the whole mass was in suspension. The manganese dioxide was dissolved by the addition of small quantities of sodium bisulfite and 30% aqueous sulfuric acid. The mixture was heated on a steam bath until the evolution of sulfur dioxide had ceased. The mixture was then cooled and extracted once with 100-ml., and twice with 50-ml., portions of ethyl ether. The ether extracts were combined and washed with water three times. The ether solution was then cautiously extracted alternately with 10% aqueous potassium carbonate solution and with distilled water. The separatory funnel containing the mixture was not agitated in the earlier stages of removal of the acidic material. After the greater part of the acidic products had been removed, the solution was agitated thoroughly in order to effect the complete removal of all acidic compounds. The ether solution was then thoroughly washed with water. All the aqueous and alkali washings were united and reextracted with ether in order to recover any traces of neutral compounds which might have been dispersed in the aqueous phase by emulsification. The ether was distilled from the united ether extracts, and the residual, fully saturated glycerides which remained were dried by heating in a vacuum and weighed.

Iodination of fractions of butterfat. The alcohol-soluble fraction and the alcohol-insoluble, acetone-soluble fraction were iodinated with Wijs solution. About 0.5 g. of the fraction was weighed into an iodine value flask and dissolved in 10 ml. of chloroform. Fifteen ml. of Wijs solution was added to the flask, and it was kept in the dark for 30 minutes. One g. of potassium iodide was then added, and the excess of iodine was titrated against 0.1 *N* sodium thiosul-

fate with starch as an indicator. The solution was then diluted with about 100 ml. of water, and the fat was extracted from the mixture with about 100 ml. ether. The ether solution was washed free from acid with water and dehydrated with sodium sulfate. The iodinated fat was freed of ether on a steam bath and finally under vacuum and the refractive index of the iodinated fat was determined at 40° C.

RESULTS

It will be noted from the data in Table 1 that the different fats showed differences in solubility when subjected to fractionation from absolute ethanol at 20° C. and subsequent separation of the alcohol-insoluble fraction from acetone at 0° C.

TABLE 1

Solubility of the fractions of different fats obtained from alcohol at 20° C. and acetone at 0° C.

Fat	Alcohol-soluble	Alcohol-insoluble	
		Acetone-soluble	Acetone-insoluble
	(%)	*(%)*	*(%)*
Butterfat	72.0	13.2	14.8
Coconut oil	98.3	—	—
Hydrogenated coconut oil	80.1	8.4	11.5
Crisco (a hydrogenated fat)	34.0	42.2	23.8
Hydrogenated cottonseed oil	28.0	48.5	23.5
Palm oil	28.0	31.9	40.1
Oleo oil	35.0	41.9	23.1
Mixture of 80% coconut oil and 20% hydrogenated fat	82.0	7.4	10.6
Synthetic fat mixture[a]	53.2	30.7	17.1

[a] The synthetic fat mixture was prepared in the laboratory from 50% rearranged lard containing 7.5% tributyrin, 25% hydrogenated coconut oil, and 25% beef tallow.

The solubility of coconut oil either as such or in a blend with hydrogenated fat was higher in alcohol as compared to the other fats. It was further observed that about 13% of total butterfat was in the acetone solution. The substitute fats other than coconut oil showed a much higher solubility in acetone, with the result that if the butterfat was mixed with 10% of any of these fats, their concentration in the acetone-soluble fraction increased two- to threefold (Table 2).

The trisaturated glyceride content of some of the typical fats and butterfat mixed with 10% of these fats is shown in Table 3. It will be noted that the addition of 10% coconut oil to butterfat increased the GS_3 content, whereas the hydrogenated cottonseed oil lowered the GS_3 content. In order to magnify the differences between butterfat and the adulterated samples, they were fractionated from ethanol, and the GS_3 content of the alcohol-soluble fractions was determined. The data are shown in Table 4.

The incorporation of 10% coconut oil in butterfat increased the GS_3 content of the alcohol-soluble fraction of butterfat significantly. This is in agreement with the fact that most of the coconut oil goes into the soluble fraction. The same holds true for hydrogenated coconut oil and a mixture containing 80%

TABLE 2

Effect of adding 10% of different fats on the solubility of butterfat in alcohol at 20° C. and acetone at 0-6° C.

Fat added:	Alcohol-soluble	Alcohol-insoluble	
		Acetone-soluble	Acetone-insoluble
	(%)	(%)	(%)
None	72.0	13.2	14.8
Coconut oil	76.8	10.6	12.6
Hydrogenated coconut oil	72.4	10.4	17.2
Crisco	67.3	17.8	14.9
Hydrogenated cottonseed oil	63.3	20.0	16.7
Palm oil	66.4	15.5	18.1
Oleo oil	67.8	16.4	15.8
Mixture of 80% coconut oil and 20% hydrogenated fat	76.0	10.0	14.0
Synthetic fat mixture	67.2	18.0	14.8

coconut oil. On the other hand, when butterfat contained any other fat, the GS_3 content was lowered.

The data in Table 5 show the changes caused in the refractive index of a fat when iodine was added at the point of unsaturation. It will be noted from the above results that the greatest change in the refractive index was caused when iodine was added to hydrogenated fat, lard, or palm oil. There was not much change in coconut oil and a mixture containing coconut oil when compared to the change caused in the refractive index of butterfat. This change was closely related to the unsaturation of the different fats.

The changes caused in the refractive indices of the fractions obtained by separation from ethanol when iodine was added to them are shown in Table 6.

It will be observed from the data that there was not much difference in the refractive indices of the original fractions. However, when iodine was added at the double bond, the differences in the refractive indices became significant. Addition of Crisco to butterfat increased the refractive indices of both the fractions of butterfat. Addition of coconut oil, however, reduced the refractive index of the two fractions. The effect of adding 10% of various fats on the refractive indices of the alcohol-soluble fraction and the alcohol-insoluble, acetone-soluble fraction of butterfat after iodination is shown in Table 7.

TABLE 3

The trisaturated glyceride content of different fats and butterfat mixed with 10% substitute fats

Fat	GS_3
	(%)
Butterfat	32.0
Coconut oil	80.0
Hydrogenated cottonseed oil	12.0
Oleo oil	14.9
Palm oil	18.2
Butterfat + 10% coconut oil	37.2
Butterfat + 10% hydrogenated fat	27.0
Butterfat + 10% oleo oil	30.3

TABLE 4

The effect of adding 10% of various fats to butterfat on the trisaturated glyceride content of alcohol-soluble fraction

Fat added	GS_3
	(%)
None	28.3
Coconut oil	34.0
Hydrogenated coconut oil	32.0
Crisco	25.8
Hydrogenated cottonseed oil	25.6
Palm oil	26.4
Oleo oil	26.1
Mixture of 80% coconut oil and 20% hydrogenated fat	31.4

The data in Table 7 indicated that the unsaturated fatty acid glycerides of a foreign fat except coconut oil were concentrated in the acetone-soluble fraction of the fat. This was reflected in a significant increase in the refractive index of this fraction after iodination, when compared with the pure butterfat fraction. The refractive index of the alcohol-soluble fraction was, however, lowered when coconut oil was added at a 10% level to the butterfat. Though the lowering is not so marked, it was a sufficient indication of adulteration.

The trisaturated glyceride content of the alcohol-soluble fraction of genuine butterfat was found to vary from 26.3 to 29.6% (Table 8). The variation of the refractive indices of the alcohol-insoluble, acetone-soluble fraction of butterfat after iodination was found to be from 1.4713 to 1.4732.

DISCUSSION

The various fats differ in the amounts of different types of glycerides present in them. It will be observed from the data in Table 9 that coconut oil is very rich in trisaturated glyceride content. The other vegetable and animal fats, however, are rich in diunsaturated and triunsaturated glycerides. The different fats that are commercially available as substitute fats can be divided into two groups from the standpoint of detecting their presence in butterfat. The first group consists of coconut oil and hydrogenated coconut oil, and the second group consists of other vegetable and of animal fats.

Some of the physical properties of a glyceride molecule, the refractive index, and the solubility are dependent on two important factors, namely, the com-

TABLE 5

The effect of addition of iodine to the fat on its refractive index

Fat	Refractive index at 40° C.	
	Original fat	Iodinated fat
Butterfat	1.4540	1.4700
Hydrogenated cottonseed oil	1.4610	1.4898
Coconut oil	1.4497	1.4520
Lard rearranged	1.4614	1.4823
Palm oil	1.4590	1.4783
Oleo oil	1.4578	1.4753
Mixture of 80% coconut oil and 20% hydrogenated fat	1.4515	1.4575

TABLE 6

The refractive indices of the iodinated fractions obtained by alcohol separation

Fraction	Refractive index at 40° C.	
	Original fraction	Iodinated fraction
Soluble fraction:		
Butterfat	1.4540	1.4713
Butterfat + 10% Crisco	1.4545	1.4741
Butterfat + 10% coconut oil	1.4535	1.4695
Insoluble fraction:		
Butterfat	1.4542	1.4689
Butterfat + 10% Crisco	1.4550	1.4720
Butterfat + 10% coconut oil	1.4540	1.4677

ponent fatty acids in the glyceride molecule and the number of double bonds or the degree of unsaturation. Fractionation of a fat from absolute ethanol selectively precipitates out high molecular weight glycerides. Butterfat is composed of a large number of glycerides because of the fact that it contains a varied number of fatty acids, particularly those of low molecular weight *(1)*. Consequently, when it is fractionated from ethanol at 20° C., about 70% of it remains in the alcohol-soluble fraction. Coconut oil was found to be almost completely soluble in alcohol, probably because of the presence of lower fatty acids in all of its molecules. The other edible fats, which are entirely made up of higher fatty acids, were found to be poorly soluble in ethanol, with the result that when butterfat is mixed with 10% of these fats they are concentrated in the alcohol-insoluble fraction. On the other hand, coconut oil is concentrated in the alcohol-soluble fraction.

The determination of the refractive indices of the two fractions obtained from alcohol separation at 20° C. represents the first step towards the detection of foreign fats in butterfat in the present method. Fractionation of the butterfat from ethanol has an advantage that it concentrates the foreign fat in one of the two fractions. The normal range of the refractive index of the alcohol-soluble fraction of butterfat was found to vary from 1.4538 to 1.4542 and that of the insoluble fraction from 1.4539 to 1.4544 *(2)*. Any deviation in the refrac-

TABLE 7

The effect of adding 10% of various fats on the refractive indices of the iodinated fractions

Fat added	Refractive index at 40° C.	
	Alcohol-soluble	Alcohol-insoluble, acetone-soluble
None	1.4713	1.4719
Coconut oil	1.4700	1.4710
Hydrogenated coconut oil	1.4698	1.4710
Crisco	1.4750	1.4774
Hydrogenated cottonseed oil	1.4749	1.4786
Palm oil	1.4725	1.4760
Oleo oil	1.4721	1.4754
Mixture of 80% coconut oil and 20% hydrogenated fat	1.4700	1.4714
Synthetic fat mixture	1.4721	1.4770

TABLE 8

The trisaturated glyceride content and the refractive indices of iodinated fractions of butterfat

Sample No.[a]	Alcohol-soluble fraction GS_3	Refractive index at 40° C. after iodination of	
		Alcohol-soluble fraction	Alcohol-insoluble, acetone-soluble fraction
	(%)		
1	27.6	1.4720	1.4728
2	28.2	1.4726	1.4730
3	28.9	1.4713	1.4721
4	26.3	1.4716	1.4725
5	27.5	1.4719	1.4732
6	28.0	1.4710	1.4723
7	29.3	1.4708	1.4713
8	29.6	1.4713	1.4720
9	26.7	1.4705	1.4719
10	27.7	1.4712	1.4721

[a] These samples had been collected from the midwestern states of the United States during the months of January, March, July, August, and December through the courtesy of H. F. Long, Sugar Creek Creamery Co., Danville, Ill., and G. A. Crapple, Wilson and Co., Chicago.

tive indices of these fractions, namely, the lowering of the refractive index of the alcohol-soluble fraction or an increase in the refractive index of the alcohol-insoluble fraction, was an indication of adulteration of butterfat with coconut oil or other vegetable and animal fat, respectively. One can predict the presence of a substitute fat in butterfat from a significant change observed in the refractive index of the two fractions. The other steps in the present method for detecting adulteration, namely, the estimation of trisaturated glycerides in the alcohol-soluble fraction and the determination of the refractive indices of the various fractions after iodination, as outlined in the procedure, may be carried out only as confirmatory tests for the detection of the substitute fats and suggested as definite proof for the purpose of prosecution in courts.

The presence of coconut oil in butterfat can be confirmed by estimating the trisaturated glyceride content of the alcohol-soluble fraction only if there is lowering of the refractive index of the soluble fraction. When butterfat was fractionated from ethanol, there was a partial concentration of trisaturated glycerides in the alcohol-insoluble fraction, thus lowering the GS_3 content of the soluble fraction. As coconut oil is almost completely soluble in alcohol, it contributes in toto the GS_3 content of the alcohol-soluble fraction. This causes a significant increase in the GS_3 content of this fraction when cocoonut oil is present

TABLE 9

Glyceride composition of representative vegetable and animal fats (molar %) (7)

Fat	GS_2	GS_2U	GSU_2	GU_3
	(%)	*(%)*	*(%)*	*(%)*
Butterfat	35	36	29	0
Beef tallow	15	46	37	2
Lard	2	26	55	17
Cottonseed oil	0	13	59	28
Coconut oil	84	12	4	0

at the 10% level in butterfat. The estimation of the GS_3 content of the alcohol-soluble fraction is thus the surest test for the detection of coconut oil alone or in a blend with other vegetable or animal fat. This test seems to be more reliable than the sterol acetate test, which is based on the differences in the melting points of vegetable and animal sterol acetates, or the Polenske value based on the component fatty acids, namely, caprylic and capric acids.

If the sample of butterfat was suspected to contain vegetable or animal fat, as seen from the increase in the refractive index of the alcohol-insoluble fraction, the iodination of the alcohol-soluble fraction and alcohol-insoluble, acetone-soluble fraction was carried out in order to confirm their presence. It was observed that the refractive index of the alcohol-insoluble, acetone-soluble fraction after iodination was significantly increased when either a vegetable or animal fat was present at a 10% level in butterfat. This is attributed to the concentration of GSU_2 and GU_3 glycerides of the foreign fat in the acetone-soluble fraction and the addition of iodine, which has a very high atomic refraction as compared to a double bond (Table 10). Not only is the iodination procedure useful in confirming the presence of a foreign fat in butter, but one can also find out whether the adulterant used was a vegetable fat or an animal fat. When an animal fat is used as an adulterant, the refractive index of the alcohol-soluble fraction after iodination is not significantly increased. The addition of vegetable fat, however, increased the refractive indices of both the fractions after iodination. Coconut oil, on the other hand, reduced the refractive indices of these fractions after iodination. It will be noted from the discussion that the method described is adequate to detect the presence of any substitute fat in butterfat at a 10% level. Moreover, one can determine which type of fat was used for adulteration.

TABLE 10

Atomic refraction of some typical groups (5)

Element or structural feature	Atomic refraction
H	1.051
C	2.501
Double bond	1.707
Cl	5.998
Br	8.927
I	14.120

SUMMARY

A method based on the glyceride structure of butterfat has been worked out to detect adulteration of the butterfat by a foreign fat at a 10% level. The suspected sample of butterfat was separated into alcohol-soluble and alcohol-insoluble triglycerides at 20° C. in order to increase the concentration of the adulterant in one of these fractions. The refractive index of the alcohol-soluble fraction of butterfat was found to vary from 1.4538 to 1.4541, whereas that of the insoluble fraction was found to vary from 1.4539 to 1.4544.

The lowering of the refractive index of the alcohol-soluble fraction indicated the presence of coconut oil. This was further confirmed by estimating the

amount of trisaturated glycerides, which was significantly increased by about 20% in this fraction by substitution of 10% of butterfat with coconut oil. The trisaturated glycerides content of this fraction of a genuine butterfat was found to vary from 26.3 to 29.6%.

The increase in the refractive index of the alcohol-insoluble fraction indicated the presence of vegetable or animal fat. This fraction was further fractionated from acetone at 0° C. The acetone-soluble fraction was iodinated with Wijs solution, and the refractive index of the iodinated fraction was determined. The refractive index of the iodinated fraction of butterfat was found to vary from 1.4713 to 1.4732. The addition of 10% of foreign fat other than coconut oil increased the refractive index significantly, enabling the detection with a fair degree of accuracy.

Limitations of the various proposed methods for the detection of foreign fat, which include the butyric acid test, sterol acetate test, tocopherol test, and the other tests based on the component fatty acids, have been discussed.

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A SUMMARY OF METHODS FOR THE DETECTION OF FOREIGN FATS IN DAIRY PRODUCTS[1]

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Adulteration of butter and fraudulent sales of imitation ice cream have been reported in the United States and Canada *(3)*. In the states that permit the production of vegetable fat frozen desserts it is reported that some ice cream is being illegally made from a blend of vegetable fat and butterfat. This process of blending vegetable or animal fats with dairy products is an open invitation to fraud and deception, since no practical test has been developed to determine the amount of these fats in dairy products. Moreover, as new techniques have been developed for modifying fats, it has become even more difficult to distinguish them from butterfat *(25)*. During the last 3 years, a number of methods have been published on means for detecting small quantities of foreign fats in butterfat. It would therefore be useful to review the various methods that have been suggested for the detection of foreign fats in dairy products.

From a chemical point of view, all fats and oils are made up almost exclusively of three units of fatty acids and one unit of glycerol and are known as triglycerides. If the acid contains only four carbon atoms, as butyric acid, its molecular weight, boiling point, and melting point are much lower than one which contains 18 carbon atoms. In addition to chain length, the degree of unsaturation also influences these properties. The combination of chain length and degree of unsaturation determines whether a triglyceride is a solid or liquid at room temperature. The significance of these differences in properties becomes apparent when they are considered from the point of view of detecting differences among different fats. Butterfat is the only edible fat which contains an appreciable amount of butyric acid. The Reichert-Meissl value is based on this property. Conversely, both animal and vegetable fats contain a predominance of 18 carbon fatty acids. Their presence would, therefore, increase the Hehner number. The Polenske value is an index of the steam-volatile and water-insoluble fatty acids, namely, caprylic and capric acids, and is useful in distinguishing coconut oil from butterfat.

Test based on butyric acid. Methods based on the butyric acid test have involved the separation of butyric acid from the mixed fatty acids of butterfat by chromatography or by steam distillation. Keeney *(14)* isolated the fat from ice cream samples by the Sager and Sanders detergent method and obtained the fatty acids by saponification with potassium hydroxide in isopropyl alcohol. Partition chromatography of the fatty acids between ethylene glycol and hexane yielded a pure butyric acid fraction and another fraction containing fatty acids

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of longer chain length than butyric acid. The butyric acid estimated by titration against alcoholic potassium hydroxide was found to vary within a narrow limit so as to permit the detection of more than 10% adulteration of butterfat.

Harper and Armstrong *(12)* obtained the fatty acids by direct saponification of the fat without extracting it from the dairy product. The butyric acid was separated by following the principle of partition and absorption chromatography with a silicic acid column and butanol-chloroform as flowing solvent. The molar concentration of butyric acid in the fat of dairy products was found to vary from 9.5 to 10%, and this index could be used for detecting fat substitution in dairy products.

Surveys on the butyric acid content of butterfat obtained from different sources in the United States over a period of more than 1 year have been made *(15)*. The extreme range of butyric acid was found to vary between 9.6 and 11.3 *M* %, and it was stated that 10% adulteration of butterfat with any fat could be detected by the butyric acid test. However, it is possible to prepare a synthetic butterfat mixture from cheap raw materials, such as beef fat, lard containing tributyrin, and coconut oil. This mixture will have the same fatty acid composition as butterfat *(1)*. It would be impossible to detect such a mixture in butterfat, particularly by the methods based on the butyric acid content of butterfat.

Test based on the urea fractionation of butterfat. Shipe *(22)* suggested a method for the identification of vegetable and animal fats in butterfat by urea fractionation. The fatty acids obtained from butterfat were fractionated by using 10% and 20% solutions of urea in methanol to form urea fatty acid complexes. The selectivity of the fractionation procedure was demonstrated by measuring the refractive indices of the various fractions. Addition of a foreign fat to butterfat caused significant differences in the refractive indices of the fractions to enable its detection. The author emphasized, however, that this is an empirical method and any deviation from the outlined procedure may alter the nature of the fractionation. Moreover, it would be difficult to detect by this method coconut oil or a synthetic fat mixture having the same fatty acid composition as butterfat.

Methods based on the unsaponifiable matter. In addition to triglycerides, most edible fats contain approximately 2% unsaponifiable matter. The unsaponifiable fraction of butterfat contains cholesterol, carotenoids, and 1 to 5 mg. tocopherol per 100 g. of fat. Methods have been proposed to detect vegetable fats in butterfat by isolating the unsaponifiable fraction and examining it for sterols or for an excess of tocopherols.

A. *Phytosterol acetate test.* Vegetable fats contain from 100 to 600 mg. of plant or phytosterols, which can readily be distinguished from the cholesterol present in butterfat by melting points of the acetate derivatives *(8)*. Pure butterfat yields a cholesterol acetate with a melting point of 115.5° C. Corn and soybean oils contain chiefly alpha sitosterol, cottonseed oil beta sitosterol, and coconut oil stigmasterol. The melting points of the acetate derivatives of these sterols are 137°, 125°, and 144° C., respectively. Addition of the vegetable fats would therefore increase the melting point of the cholesterol acetate extracted from the but-

terfat. This method, however, cannot be used to detect oleo oil, beef tallow, or lard in butterfat since these animal fats contain approximately the same amount of cholesterol as butterfat. Furthermore, the method would not be successful when applied to a mixture of 10% hydrogenated coconut oil and 90% butterfat, since coconut oil contains less than 100 mg. of total phytosterols.

B. *Test based on the tocopherol content of butterfat.* Another component of the unsaponifiable fraction, namely, tocopherol, has recently been suggested as a basis for a test for detecting foreign fats in butterfat. The latter contains only a few milligrams of tocopherol whereas vegetable fats contain approximately 100 mg. of tocopherols per 100 g. fat. Therefore, if vegetable fat is used as the adulterant, its presence would be easily detected.

Alpha tocopherol is the principal form present in animal fats, whereas vegetable fats contain considerable quantities of nonalpha forms. Shipe *(23)* developed a method to determine the concentration of the nonalpha form colorimetrically. The presence of a detectable amount of the nonalpha form in dairy products was claimed to serve as evidence of adulteration with vegetable fats.

Anglin *et al. (2)* developed a rapid procedure for the determination of total tocopherol in butterfat after removal of the interfering materials, such as oil yellow OB and vitamin A, and applying a correction factor for carotene. The color was developed in the extracted fat with ferric chloride and 2,2[1] bipyridine reagent and was measured colorimetrically. Butterfat with tocopherol values of more than 50 γ per gram fat was considered to be adulterated on the basis of the tocopherol values. However, a mixture of animal fats and coconut oil can be prepared which cannot be detected by the procedure of either Shipe or Anglin *et al.*

Test based on the unsaturated fatty acids. Methods based on the unsaturated fatty acid content of butterfat also have been suggested by various workers for the detection of butterfat adulteraton. Venkatasubramanian and Banerjee *(26)* developed a method for the detection of butterfat adulteration by estimating its linoleic acid content. They observed that butterfat did not contain more than 2% linoleic acid whereas vegetable oils and hydrogenated fats contained a much higher percentage of this acid. If a sample contained these fats, the linoleic acid content increased, enabling their detection in butterfat. However, hydrogenated coconut oil, oleo oil, and lard cannot be detected by this method since these fats contain less than 2% linoleic acid.

Ambrosetti *(1)* developed a test based on iso-oleic acid content to detect adulteration with a hydrogenated fat. In this method, the mixed fatty acids from a sample of suspected butterfat were separated into solid and liquid fractions by the lead salt separation method. The lead salt of iso-oleic acid, which is formed during the hydrogenation of fats, is alcohol-insoluble and precipitates with the solid fraction. The solid fraction was further recrystallized and treated with iodine. The iodine used up was calculated to iso-oleic acid. Ambrosetti found that no more than 1.3% of twice recrystallized iso-oleic acid was found in authentic butterfat. Hydrogenated porpoise oil contained 7 to 12% iso-oleic acid, depending upon the manner of hydrogenation. Adulteration with hydrogenated

oil could be detected if the iso-oleic acid content of the fat was higher than 1.5%. The infrared spectra of fats can also be used to detect adulteration if hydrogenated fats are suspected, but this method cannot be used to detect oleo oil *(18)*.

A method based on ultra violet spectrophotometric analysis has been devised by Lembke *et al. (19)*. Margarine has three absorption peaks in the triene range as compared to two absorption peaks for butterfat. In the tetraene range, butterfat shows maximum absorption at 3020 Å and 3170 Å, whereas margarine is lacking in absorption at 3020 Å. Based on this difference, a method was developed that was claimed to be capable of distinguishing butter from butter containing margarine. With increasing margarine content, the difference in heights expressed in millimeters between absorption at 3020 Å and that at approximately 3000 Å decreased. However, this method cannot be used to detect coconut oil, lard, or oleo oil.

Test based on iso-valeric acid content. Fabris and Vitagliano *(10)* suggested a chromatographic identification of hydrogenated dolphin oil in butterfat. They observed that dolphin oil contains iso-valeric acid, which is not found in butterfat. The presence of 2.5% hydrogenated dolphin oil in butterfat can be detected by paper chromatography by using butanol saturated with water as the liquid phase and ammonia as the gas phase.

Hydroxamic color test. Nelson *(20)* developed a color test that can be used to distinguish butterfat from other animal fats and from plant fats. The test was based on the formation of pink colored hydroxamic acid complexes from the esters of the lower fatty acids of butterfat. The complexes formed from other fats (except coconut oil) did not contribute to the distinctive color.

Test based on the addition of indicator substances to hydrogenated fats. The addition of indicator substances to hydrogenated fats has been suggested from time to time as a means of detecting these fats in butterfat. The addition of vegetable coloring matter such as chlorophyll, carotene, alkanaet, kamala, rantanjot, and turmeric to hydrogenated fats has been recommended *(24)*. The available data, however, indicate that all of these coloring materials were easily removed by treatment with animal charcoal *(21)*.

The addition of raw sesame oil *(9)* to vegetable oil products to the extent of not less than 5% insured their detection by the Baudouin test at a 10% level in butterfat. The Baudouin test is considered to be specific for sesame oil. The test consists of shaking the fat with a solution of sugar and adding an equal volume of concentrated hydrochloric acid. If sesame oil is present, a crimson color develops after the mixture is allowed to stand for 10 minutes. The intensity of the color is, however, reduced by treatment of the sesame oil with animal charcoal or fuller's earth and is also reduced in refined and deodorized sesame oil. Of all the chemicals tried in the category of latent colors, Subramanian *et al. (24)* have found that phenolpthalein is by far the best for the purpose of detection. It can be incorporated at a level of 10 mg. % in fat either in alcoholic solution or direct dissolution in hot hydrogenated fat. At this concentration of phenolpthalein, the hydrogenated fat can be easily detected by shaking the fat with an alkali. Furthermore, as phenolphthalein is soluble in fat, it cannot be removed

by treatment with animal charcoal or fuller's earth. On repeated extraction with warm alkaline solution the fat can be freed of added phenolpthalein. The process is, however, tedious and impracticable.

Bryant and Biggs *(7)* observed that vegetable fats containing 0.02% butylated hydroxyanisole could be easily detected at a 10% level in butterfat. Gulati and Kartha *(11)* have shown that ethyl tyrosinate may be used as a tracer in the detection of butterfat adulterated with hydrogenated fat. Ethyl tyrosinate was added to the hydrogenated fat at a level of 0.005%. Adulteration of butterfat with 5% of such fat could be easily detected by following the Ehrlich's diazo test. The fat was extracted with 1:1 hydrochloric acid and the esters were hydrolyzed by refluxing the extract for 30 to 60 minutes. It was then treated with a freshly diazotized solution of 0.25% 4-aminobiphenyl in hydrochloric acid. The mixture was made slightly alkaline with sodium carbonate solution. If tyrosine was present, a red color developed within 5 minutes.

Methods based on the glyceride composition of butterfat. Methods based on differences in the properties of triglycerides have been suggested as a means of detecting adulteration of butterfat with a foreign fat.

A. Method based on fractionation of butterfat by selective solidification. Krienke *(17)* has suggested fractionation by selective solidification as an aid in detecting butterfat adulteration. The fat was fractionated by a process of partial solidification and filtration at successive temperatures of 95°, 80°, 70°, and 60° F., and the Reichert-Meissl value of these fractions was determined. Krienke claimed that the differences in Reichert-Meissl values between pure and adulterated butterfat indicated positive identification of adulteration at levels as low as 2% of foreign fat. This method is useful, but it may be possible to duplicate the solidification rate by a proper mixture of various edible fats.

B. Presumptive crystallization test. Keeney *(16)* developed a presumptive crystallization test for the detection of milk fat adulteration. The fat was dissolved in an alcohol solvent (mixture of isopropyl alcohol and ethanol) at 45° C. The author claimed that the properties of the crystals, which precipitated out when the mixture was cooled to 25° C., served to identify the fat used in adulteration. As edible fats contain various polymorphic forms, it may not be easy to control crystallization and to obtain the desired polymorphic crystals.

C. Critical temperature of dissolution of butterfat. According to Bhide and Kane *(6)*, gross adulteration of butterfat with hydrogenated fats can be easily detected by determining the critical temperature of solution for the butterfat in a solvent mixture of ethanol and isopropyl alcohol. This test is essentially qualitative and good only for the elimination of highly adulterated samples of butterfat.

D. Method based on the fractionation of butterfat from absolute ethanol. Bhalerao and Kummerow *(4)* suggested a method that is dependent on differences in the solubility properties of triglycerides in absolute ethanol. The butterfat was separated into alcohol-soluble and alcohol-insoluble triglycerides at 20° C. from absolute ethanol in order to increase the concentration of the adulterant in one of the fractions. The addition of 10% coconut oil lowered the refractive

index of the alcohol-soluble fraction, whereas the addition of any other fat increased the refractive index of the alcohol-insoluble fraction. Any deviation in the refractive indices of the butterfat fractions indicated the presence of a foreign fat in the butterfat. The differences in the refractive indices caused by the addition of 10% of substitute fats prepared from mixtures of coconut oil and animal fats may, however, be too small for a positive sign of adulteration.

A further refinement in the method was, therefore, carried out by the authors in order to detect all possible combinations of foreign fats in admixture with butterfat *(5)*. The initial fractionation of the sample indicated the type of the adulterant present. If the sample was suspected of containing coconut oil, the trisaturated glyceride content of the alcohol-soluble fraction was determined. The trisaturated glyceride content of a pure butterfat fraction was found to vary from 26.3 to 29.6% and was found to be significantly increased when coconut oil was used as an adulterant. If the sample was suspected of containing animal fat or vegetable fat other than coconut oil, the alcohol-insoluble fraction was further fractionated from acetone at 0° C. The acetone-soluble fraction was iodinated by using Wijs' solution, and the refractive index of the iodinated fraction was determined at 40° C. The refractive index of this fraction was found to vary from 1.4713 to 1.4732 in pure butterfat and was found to be significantly increased by the addition of 10% of a foreign fat other than coconut oil.

E. Method based on the estimation of azelaoglycerides of butterfat. The different types of glycerides in a fat can be estimated by oxidizing it with potassium permanganate and acetic acid according to the method outlined by Kartha *(13)*. The addition of a substitute fat to butter will cause differences in the proportions of azelaoglycerides formed during the oxidation from those of authentic butterfat. However, beef fat, hydrogenated vegetable oil, and coconut oil can be mixed in such a proportion as to duplicate the glyceride structure and the azelaoglycerides that can be obtained from pure butterfat. This method, therefore, cannot be used to detect such a mixture.

Some of the important methods that have been proposed for the detection of foreign fats in butterfat have been applied to deliberately adulterated samples in this laboratory. The results of these analyses are reported in Table 1. It will be noted from the data in the table that the method based on the glyceride structure is the only useful one for detecting adulteration of butterfat with 10% of any substitute fat. The methods based on butyric acid content and the unsaponifiable fracion of butterfat cannot detect a synthetic fat mixture having almost the same fatty acid composition as butterfat. Moreover, the method dependent upon the unsaponifiable matter is not useful for detecting animal fat or coconut oil.

It appears from the above discussion that one cannot rely on determinations based on either the mixed fatty acid composition or the unsaponifiable fraction as a method suitable for detecting the presence of all conceivable mixtures of substitute fats. However, butterfat has a characteristic glyceride structure, and to date it is not possible to duplicate a fat with the exact glyceride components that are present in butterfat.

TABLE 1

Limitations of the various methods suggested for detecting adulteration of butterfat at 10% level

Method	Butterfat	Variation for butterfat	Butterfat + coconut oil	Butterfat + Crisco	Butterfat + oleo oil	Butterfat + synthetic fat[a]	Butterfat + mixture[b]	Butterfat + rearranged lard[c]
Butyric acid method *(14) (M %)*	10.1	9.6-11.3	9.3	9.2	9.0	10.0	9.3	10.1
Tocopherol method *(2) (γ per g. fat)*	31.6	up to 50 γ per g. fat	39.4	166.0	31.5	31.6	71.0	32.0
Phytosterol acetate method *(8)*	113.5° C.	113-116	115.1° C.	121.6° C.	114.0° C.	114.3° C.	117.6° C.	113.2° C.
Critical temp. of dissolution method *(6)*	44° C.	42-46° C.	40° C.	47° C.	46° C.	44° C.	41° C.	46° C.
Polenske method	1.8	1-2.6	2.9	1.6	1.6	2.3	2.6	1.6
Glyceride structure:								
A. Trisaturated glycerides of alcohol soluble fraction of fat *(%)*	28.5	26.3-29.6	34.2	25.8	26.1	29.0	32.6	26.3
B. nD at 40° C. of alcohol-insoluble acetone-soluble fraction of fat after iodination *(5)*	1.4732	1.4713-1.4732	1.4700	1.4800	1.4785	1.4783	1.4746	1.4780

[a] Synthetic fat mixture of 25% coconut oil + 25% beef fat + 50% lard rearranged with 7.5% tributyrin.
[b] Mixture of 75% coconut oil + 25% hydrogenated cottonseed oil.
[c] Lard rearranged with 7.5% tributyrin.

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COMPARISON OF BAKING QUALITY OF FROZEN CONDENSED AND SPRAY-DRIED SKIMMILK

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Previous investigations of frozen condensed skimmilk containing 27% solids *(2)* indicated that this product could be satisfactorily used in bread if the milk had received adequate heat treatment prior to freezing. Various amounts of sucrose were added to this 3:1 skimmilk, and although they improved the body of the thawed samples, neither the sugar nor the storage temperature was an important factor in determining the baking quality of the thawed skimmilk. Heat treatment of 82° C. for 30 minutes proved adequate, since samples so heated produced dough and bread of superior characteristics.

In these earlier investigations of the baking quality of frozen condensed skimmilk, the dough quality and loaf volumes were compared with those obtained when commercially produced dried nonfat milk solids, of good baking quality, were included in the bread formula at equivalent levels.

The purpose of the present investigation was to compare the baking quality of frozen condensed skimmilks having a solids ratio higher than 3:1 (27% nonfat milk solids) with that of spray-dried skimmilk obtained from another portion of the same skimmilk. Since it is known that denaturation takes place more rapidly and to a greater extent in frozen milks having a higher solids content than 27% *(1)*, greater destabilization would be expected after a comparable storage period than at the lower solids level.

METHODS AND MATERIALS

Whole milk was heated to 43° C. and separated. Part of the resultant skimmilk was held at 63° C. for 30 minutes (low-heat treatment), cooled to 49° C., and condensed to 36% solids. A portion of this condensed skimmilk was packaged in 1-l. cans and placed in a storage room at −17° C. The remainder was spray-dried, stored at 4° C., and used as the low-heat–treated skimmilk powder.

The remaining raw skimmilk was held at 82° C. for 30 minutes (high-heat treatment), cooled to 49° C., and condensed to a 4:1 milk-solids ratio. A portion of this skimmilk, which now contained 36% milk solids-not-fat, was withdrawn from the pan, placed in 1-l. cans, and stored at −17° C. Another portion was spray-dried and stored at 4° C. for later use in the baking trials. The skimmilk containing 36% milk solids, which remained in the vacuum pan, was condensed further until a 5:1 solids-ratio product, containing 45% milk solids-not-fat, was obtained. Cans of this product were placed in storage at −17° C. A can of each milk prepared as described was removed periodically over approximately a year from each storage space and the contents were thawed in a water bath at 21° C. After thawing, the cans were opened, their contents were poured into a suitable

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container, and the physical appearance of the product was noted. After moderate mixing, a portion was reconstituted to 9% solids and used to evaluate flavor and in determining the solubility index expressed as milliliters of sediment per 50 ml. of sample. In determining the latter, a 50-ml. portion was placed in a graduated conical tube and centrifuged at 1,000 r.p.m. for 5 minutes. The maximum desirable sediment in stable milks was considered to be 0.5 ml.

RESULTS AND DISCUSSION

The day after the condensed skimmilks were prepared, the viscosity values at 22° C., as determined with a MacMichael viscosimeter, were: for skimmilk having a 4:1 solids ratio and a low-heat treatment, 172 cps.; for 4:1 high-heat–treated skimmilk, 480 cps.; and for 5:1 high-heat–treated milk, 28,690 cps. The high-heat treatment caused an increase in the viscosity of the 4:1 milk. This comparison could not be made on 5:1 milks, since no low-heat treatment product containing 45% milk solids-not-fat was prepared. As expected, the high-heat–treated skimmilk had a heated flavor, and a condensed flavor was noted in all of the condensed products.

Since it was desired to obtain destabilized milks for baking purposes, samples were stored 2 months before examination. At this time all thawed samples were heterogeneous and their viscosities could not be determined accurately. They were semi-solid and were described as dry and resembling cold mashed potatoes. In the 4:1 milk there was some whey separation. The flavor of all samples, after reconstituting to 9% total solids, was bland. The 5:1 samples were gritty owing to crystallization of lactose.

At 90 days, the thawed samples were about the same except that they were becoming firmer and the 4:1 samples were also becoming gritty. Free whey was beginning to appear throughout each sample. Sediment content of the reconstituted milks was measured at this examination. It was found that even after storage at –27° C. the 5:1 milk had 12 ml. of sediment per 50 ml. of reconstituted sample. This was more than that found in either the low- or high-heat–treated 4:1 milks. However, it was less than that observed in the 5:1 samples stored at –17° C. The flavor of all milks remained bland and continued so throughout the entire experiment.

At the end of 240 days, the 4:1 samples were becoming pasty, and more wheying-off was observed than in previous examinations. The 5:1 sample that was stored at –17° C. was dry and crumbly; the same milk stored at –27° C. also was crumbly but did not possess the dry appearance of the milk stored at –17° C. The quantity of sediment (10 ml.) from all milks was about the same at this examination.

From the end of the ninth month (270 days) until completion of the experiment (1 year), no particular change was noted that was not observed at the eighth-month examination, except for differences in the quantity of sediment. These were probably due to the rapid rate at which settling occurred after shaking and prior to pouring into the 50-ml. conical centrifuge tubes. The solubility

of those milks became less and less as storage time increased. They were in poor physical condition and were wholly undesirable from the beverage standpoint.

In order to compare the baking quality of the variously treated and stored skimmilk samples, experimental baking tests were carried out on the fresh samples and on the stored samples at appropriate time intervals over a period of 1 year.

The flour used in the experimental baking test was an equal mixture of commercially produced bakers' grade patent, spring wheat (Northwestern origin), and winter wheat (Southwestern origin), which were selected to provide a flour mixture corresponding to flour that would be used in commercial bakeries. All other ingredients in the baking formula were also of commercial type and grade. The amounts of various ingredients, considered typical of customary bread formulas, are given below:

	Parts by weight
Flour	100
Sugar	5
Salt	2
Lard	2
Malted wheat	½
Yeast food	¼
Yeast	2
Nonfat milk solids	6
Water—variable to give proper dough consistency	

The straight dough procedure of bread manufacture was followed, and the dough mixing was carried out in a Fleischmann laboratory mixer at time intervals necessary for proper development of the dough. Two dough pieces, each sufficient for a 1-lb. loaf of bread, were obtained from each mixing. Fermentation was carried out in closed containers in a constant-temperature room held at 28° C.; kneading intervals followed 1 hour and 35 minutes, 40 minutes, and 20 minutes. The dough was proofed at 35° C. and 92% humidity for 60 minutes. Bread was baked in a 4-shelf, reel oven, thermostatically regulated to 204° C. for 30 minutes. The loaf volumes were obtained on the day following the baking, and the grain, texture, and crumb characteristics were noted. Since these characteristics could not be measured with reliable accuracy, only loaf-volume measurements are presented for comparison of the baking quality of the milk solids used in the bread formulas.

Except for the amount of water added, all conditions of bread manufacture and bread formula ingredients were kept constant, and no variations were made to bring about conditions which might yield more satisfactory bread with milk samples of inferior baking quality. Extra water, equal to the weight of the skimmilk solids, was added to doughs containing skimmilk that had been held at 82° C. for 30 minutes. This extra water was not necessary in doughs containing skimmilk that had been held at 63° C. for 30 minutes. At each baking a separate dough was included that contained no milk solids but was identical in all other

TABLE 1

Influence of storage time on loaf volume obtained with nonfat milk solids prepared and stored under various conditions (Four-loaf average expressed in ml.)

Months in storage	Water control	Low-heat powder (62° C/30′)	High-heat powder (82° C/30′)	Low-heat froz. cond. (4:1/−17° C.)	High-heat froz. cond.		
					(4:1/−17° C.)	(5:1/−17° C.)	(5:1/−27° C.)
0	2367[a]	2375	2419	2275	2506	2500	2500
2	2281	2425	2481	2306	2525	2419	2463[a]
3	2413[a]	2356	2544	2225[a]	2494	2525	2531
4	2512[a]	2312[a]	2512[a]	2267[a]	2475[a]	2512[a]	2512[a]
7	2463	2463	2469	2169	2488	2369	2450
9	2393	2275	2450	2169	2350	2413	2413
12	2538	2325	2544	2138	2581	2494	2569
			Loaf volume average over one-year storage				
	2422	2365	2502	2217	2489	2458	2492
			Variations in loaf volume averages from total average				
0	−55	+10	(−83)	+58	+17	+42	+8
2	(−141)	+60	−21	(+89)	+36	−39	−29
3	−9	−9	+42	+8	+5	+67	+39
4	(+90)	−53	+9	+50	−14	+54	+20
7	+41	(+98)	+66	−48	−1	(−89)	−42
9	+49	(−90)	−53	−48	(−139)	−45	(−79)
12	(+116)	−40	+41	(−79)	(+92)	+36	(+77)

[a] Two-loaf average.

respects to the other doughs baked at that time. This so-called "water dough" was carried through the standard procedure, and the loaf volume was obtained in order to observe any changes in baking which might be due to causes other than the nonfat milk solids.

The measurement of the baking quality of the various milk samples was limited in this investigation to measurement of loaf volume of the bread produced when the milk was included in the bread formula at the level of 6 parts milk solids per 100 parts flour. However, the properties of the dough during the bread production and the characteristics of the bread crumb are equally important. Throughout the duration of the experiment, the dough characteristics, as well as the grain and texture of the bread crumb, were constant, depending upon the treatment of the milk samples. Without exception, the low-heat–treated, frozen condensed skimmilk and its corresponding dried skimmilk produced dough of inferior quality in the sense that it was stiff, nonpliable, putty-like, and required long mixing; the crumb of this bread invariably was harsh-feeling and was comprised of irregularly shaped cells with thick walls. When high-heat–treated milk samples, either frozen or powdered, were used in the bread formula, the reverse characteristics were always noted.

In Table 1 are shown the loaf volume averages obtained when fresh and stored skimmilk samples were included in white pan bread at 6% level of milk solids based on flour weight. Also included in Table 1 are the grand average loaf volumes (calculated from each original loaf volume measurement) for a given milk over the entire duration of the experiment and the differences between this voume and the averages for specific storage periods. The standard error of a single determination in this series was approximately 75 ml., and variations in loaf volume average above this figure have been indicated by parentheses. It is seen that there is no recognized trend for any sample to become progressively inferior or superior to the average value of that variable for the entire experiment. This is interpreted to mean that none of the variously prepared and stored skimmilk products changed materially over the period of the experiment in their baking quality as measured by loaf volume.

Several interesting relationships can be recognized from Table 1. High-heat, nonfat, dried milk solids included in bread formulas not only produced substantially larger loaf volumes than did the low-heat–treated dried milk solids (a fact which has been recognized for some time) but also produced slightly larger loaf volumes than identical milk solids preserved in a frozen state under any of the conditions of temperature or concentration that were used. It is also shown that low-heat–treated dried skimmilk solids invariably produced larger loaf volumes than did identically treated nonfat milk solids preserved in concentrated frozen state at –17° C. during a year, but not as large as any of the loaves that contained solids from high-heat frozen condensed skimmilk.

SUMMARY

Skimmilks condensed to 4:1 and 5:1 became extremely unstable when stored for long periods at –17° and at –27° C. These milks developed high viscosity,

poor texture, and undesirable appearance. The high-heat treatment caused higher viscosities in otherwise similar skimmilks. The lower (–27° C.) storage temperature delayed the rate of denaturation of the condensed skimmilk. These milks were entirely unsatisfactory from the beverage standpoint.

The temperature of storage caused no improvement or impairment in baking quality as determined by dough characteristics, texture of crumb, and loaf volume of bread. High-heat–treated (82° C. for 30 minutes) skimmilk solids, preserved in a frozen or dried state, produced larger loaf volumes than corresponding low-heat (62° C. for 30 minutes) skimmilk solids. Heat treatment was the primary factor in determining baking quality. The slightly greater loaf volumes obtained with skimmilk solids stored in a dried instead of in a frozen condensed state indicate some advantage in preserving skimmilk solids in this form.

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STABILITY OF SMALL CONCENTRATIONS OF PENICILLIN IN MILK AS AFFECTED BY HEAT TREATMENT AND STORAGE[1]

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Various antibiotics have been used extensively as therapeutic agents against bovine mastitis and as feed supplements. The treatment of mastitis with antibiotics has presented a problem in dairy technology, since small quantities of antibiotics which may appear in milk from treated cows greatly impede the lactic acid fermentation desired in the manufacture of cheese and other cultured dairy products.

The occurrence of antibiotics in milk from treated cows has been well established. However, little has been reported regarding their stability as affected by various heat treatments and storage conditions. Foster and Woodruff *(6)* have reported that 5 to 30% loss of potency resulted when they pasteurized the penicillin broth at 65° C. for 30 minutes. Hunter *(9, 10)* has reported 0 to 11% loss of penicillin in milk upon pasteurization at 143° F. for 30 minutes and 73% upon autoclaving. On the other hand, Doan *(4)*, Krienke and Fouts *(12)*, and Hood and Katznelson *(8)* observed that pasteurization does not influence appreciably the inhibitory effect of penicillin on the acid production by starter cultures. Krienke did not find any detectable loss of penicillin potency upon 9 to 10 days storage of whole milk and condensed milk and upon 10 weeks storage of skimmilk powder. Kersey *et al. (11)* in determining the preservative effect of antibiotics in foods observed that the added antibiotics were destroyed when the foods were cooked. They observed eight antibiotics to be stable in milk held at 8° to 10° C. for at least 1 week.

In almost all the above studies the amount of retardation in acid development by active starter has been taken as a criterion for determining the quantity of the antibiotic present. However, this method is not specific for antibiotics, nor is it quantitatively sensitive, since acid inhibition and quantity of the antibiotic are not always directly related.

The present investigation was undertaken to determine systematically the effect of various heat treatments and storage conditions upon penicillin in milk by using a quantitatively sensitive method for penicillin assay and by using different kinds of penicillin. Similar studies with streptomycin and chlortetracycline (aureomycin) were reported previously *(14, 15)*.

EXPERIMENTAL PROCEDURE

Attention was given to methods of determining quantitatively small concentrations of penicillin. Attempts to adapt colorimetric methods of Scudi *(13)* and

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Boxer and Everett *(2)* were unsuccessful, since these methods, originally devised for pure penicillin powders and penicillin broth, could not be applied to the extremely low concentrations of penicillin generally found in milk.

Several bacteriological methods reported in the literature *(5, 12, 17)* were investigated, and the disk assay method suggested by Silverman and Kosikowsky *(16)* and by Difco Laboratories *(3)* was found to be accurate and sensitive and was used throughout this investigation with slight modifications.

The method described *(3)* required the preparation of the assay petri dishes by pouring about 8 to 10 plates per 100 ml. of whey agar seeded with one ampule of *Bacillus subtilis* spore suspension. However, for the purpose of this study, 10 ml. of seeded agar were pipetted into each special flat-bottom petri dish (Corning #3162) in order to standardize thickness of agar layer in each plate.

The principle of this method is that a paper disk containing antibiotic is placed on a hardened agar layer in a petri dish. The agar is previously inoculated with a sensitive test organism for penicillin. The plates thus prepared are incubated. During incubation the antibiotic diffuses radially, and a circular zone of inhibition is formed. The diameter of the zone of inhibition is related directly to the concentration of antibiotic in the sample.

Studies were conducted to determine the temperature and the combinations necessary to completely inactivate penicillin in milk. Fresh milk was inoculated with 0.2-0.5 I.U. of K penicillin per milliliter. These small concentrations were used in order to simulate conditions that might prevail in the milk from treated animals. The inoculated milk was mixed thoroughly and heated in test tubes in a thermostatically controlled water or oil bath. An autoclave was used for heating milk at 250° F. Heating of inoculated milk was carried out at 160°, 190°, 200°, and 250° F. for various lengths of time. The processed samples were then assayed for antibiotic activity by two methods. One method was the disk assay method described above and the other was the starter inhibition method. The starter inhibition method was carried out by inoculating the milk and a control with 5% active starter and comparing the acid development after 15-16 hours of incubation at 35° C. The control milk sample, known to be free from antibiotics, had been heated for the same length of time as the test samples. Negative results by both methods were used as the criterion for the complete inactivation of penicillin.

In the study of the effect of various heat treatments and aging, fresh raw milk, 1% phosphate buffer (pH 6.0) and distilled water (pH 6.6) were inoculated with freshly prepared penicillin solution in concentrations from 0.09 to 1.07 I.U. per milliliter. The inoculated product was mixed thoroughly and divided into two lots. One lot was held raw and the other was laboratory pasteurized in screw-capped test tubes in a thermostatically controlled water bath.

Since, for the same concentration of antibiotic, comparatively smaller zones were developed for milk than for water or buffer, standard curves were prepared for each medium. Hammond *(7)* also observed that dihydrostreptomycin in milk produced a smaller zone than did the same concentration in serum.

RESULTS AND DISCUSSION

Thermal inactivation of K penicillin. The data obtained in the study to determine the thermal inactivation of penicillin in milk have been plotted in Figure 1. Each point represents an average of 3 or 4 trials. The results obtained

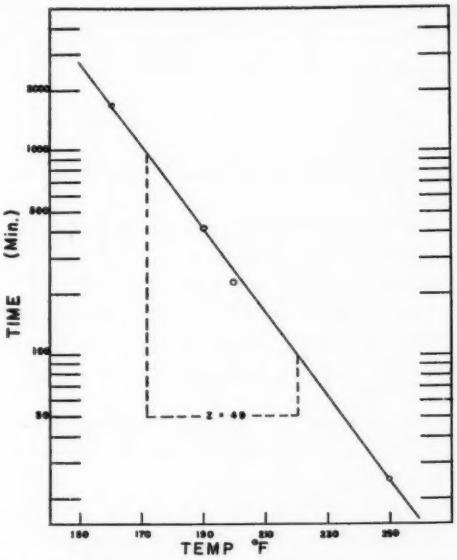

FIG. 1. Thermal inactivation of K penicillin in milk.

in the various trials did not vary considerably. To inactivate penicillin completely, an average of 25, 230, 420, and 1,705 minutes heat treatment was necessary at 250°, 200°, 190°, and 160° F., respectively. The results when plotted semilogarithmically followed a straight line, suggesting that the thermal inactivation of penicillin may follow the monomolecular law within the range of temperatures studied. By using the method suggested by Ball *(1)*, the Z value for the thermal inactivation curve for penicillin in milk was found to be 49.0. It is significant to note that by extrapolation one could determine theoretically various time and temperature combinations for thermal inactivation of the antibiotic.

Effect of pasteurization on different kinds of penicillin. To determine the effect of pasteurization on different kinds of penicillin added to milk, raw milk was inoculated with five different penicillin preparations and then pasteurized at 143° F. for 30 minutes. Data are presented in Table 1. The data reveal that an average of 10.7% loss of potency occurred when the penicillin-milk was pasteurized and that the different kinds of penicillin varied greatly in their heat stability. However, there were wide variations among individual trials. Potassium penicillin 0 appears to be the least susceptible to heat with a loss of only

TABLE 1
Effect of pasteurization (143° F.–30 minutes) on different kinds of penicillin in milk

Penicillin source	No. of trials	Penicillin inoculated I.U./ml (Range)	% loss of potency	
			Range	Average
Potassium penicillin O	4	0.19-0.50	−2.0 to 5.3	0.9
Procaine penicillin	4	0.37-0.94	1.4 to 10.6	7.6
Penicillin K	3	0.17-0.45	5.9 to 15.6	10.9
Penicillin G salt	4	0.32-0.82	6.3 to 21.9	13.1
Ephenamine penicillin G	8	0.09-0.90	0.0 to 43.3	16.1
			Average	10.7

0.9%, whereas ephenamine penicillin G was the most heat-sensitive with an average potency loss of 16.1%.

Effect of various heat treatments and aging on K penicillin. Raw milk, buffer, and water were inoculated with K penicillin, and the effect of the following time and temperature combinations was investigated: *(a)* 143° F. for 30 minutes, *(b)* 160° F. for 30 minutes, and *(c)* 250° F. (autoclaving at 15 lb.) for 15 minutes. Penicillin determinations were made on raw and processed samples immediately and at 24-hour intervals for 7 days of storage at 34°-38° F. Results are presented in Table 2.

The data revealed that there was a direct relationship between the temperature and the penicillin inactivation and that the antibiotic was heat-labile in an increasing order in milk, buffer, and water.

Heating the milk containing penicillin at 143° F. for 30 minutes, at 160° F. for 30 minutes, and at 250° F. for 15 minutes produced an average loss of antibiotic potency of 8.2, 10.1, and 59.7%, respectively. Penicillin was less heat-stable in buffer than in milk and 28.9%, 34%, and 100% of the antibiotic was destroyed by the 143°, 160°, and 250° F. heat treatment, respectively. The antibiotic was found to be more heat-labile in water than in milk or buffer. More than half of the penicillin activity was lost upon heating the water at 143° F., and the antibiotic was inactivated completely when heat treatments were at 160° or 250° F.

In Table 2, cumulative losses of penicillin upon heating and during subsequent storage are expressed in terms of per cent of the original concentration of penicillin added to the products. The antibiotic was found to lose activity in raw and processed samples during storage at 34°-38° F. When raw milk containing penicillin was stored, an average of 46.6% loss of potency resulted in 6 days. After 6 days, antibiotic activity could not be determined accurately in those samples, since at the immediate periphery of each assay dish and within the clear zone of inhibition a heavy ring of bacterial growth developed.

The organism responsible for such growth was identified as a Gram-negative short rod, which grew profusely on a nutrient agar slant and in nutrient broth. It was a gas-former, facultative aerobe and nonproteolytic. It was a very mild acid producer. Penicillin had neither stimulating nor inhibiting effect on its growth. The organism could grow in broth or skimmilk containing up to 75

TABLE 2

Loss of K penicillin potency in milk, buffer, and water resulting from heat treatment and storage

Heat treatment	No. of trials	Penicillin concentration added U/ml	Total loss of penicillin upon heating and during subsequent storage. At the end of				Storage loss only in 7 days Calculated on bases of	
			Heating	1 day	3 days	7 days	Penicillin added	Penicillin at start of storage
			(%)					
Milk								
Raw	7	0.13-0.53	—	9.6	28.7	46.6[a]	46.6	46.6
143° F.—30 min.	8	0.13-0.58	8.2	13.8	28.2	43.0	34.8	37.9
160° F.—30 min.	6	0.25-1.00	10.1	20.0	24.5	30.7	20.6	22.9
250° F.—15 min.[b]	6	0.25-1.00	*59.7	62.0	62.9	69.6	9.9	24.5
Buffer								
Raw	6	0.28-1,07	—	13.9	18.4	29.7	29.7	29.7
143° F.—30 min.	4	0.28-0.96	28.9	35.8	45.6	53.7	24.8	34.9
160° F.—30 min.	2	0.57-1.07	34.0	38.4	42.0	50.8	16.8	25.4
250° F.—15 min.[b]	2	0.52-1.04	100.0	100.0	100.0	100.0	—	—
Water								
Raw	6	0.28-1.07	—	19.3	24.3	42.1	42.1	42.1
143° F.—30 min.	4	0.28-0.96	54.7	69.0	77.0	86.3	31.6	69.7
160° F.—30 min.	2	0.57-1.07	100.0	100.0	100.0	100.0	—	—
250° F.—15 min.[b]	2	0.52-1.04	100.0	100.0	100.0	100.0	—	—

[a] Loss of potency in 6 days.

[b] Autoclaved at 15 lb. for 15 min.

units of penicillin per milliliter. It was highly heat-labile and was killed within 5 minutes at 143° F. in sterile skimmilk.

In the processed milk samples, loss upon heating and storage for 7 days was observed to be 43.0%, 30.7%, and 69.6% in the samples heated at 143°, 160°, and 250° F., respectively. The unheated and heated buffer and water samples exhibited a pattern of losses upon storage similar to that of the milk samples.

In the last two columns in Table 2 are presented only the storage losses, calculated as the per cent of the original concentration added to the products (column 8) and also as the per cent of the amount of penicillin present at the start of storage (column 9). In milk samples heated at 143° and 160° F. the storage losses calculated by both methods differ very little, since the heat losses are very small. However, this was not so in the milk samples heated at 250° F. or the heated buffer and water samples, all of which showed considerably greater losses upon heating.

In general, during storage the antibiotic loss in milk was greater in raw samples than in the heated ones and was less in buffer than in the other systems. Within the limits of penicillin concentrations studied, there was no relationship between the concentration of the antibiotic and the rate of inactivation due to heat treatment or storage.

SUMMARY

The thermal inactivation of penicillin in milk was determined at various temperatures. The results obtained when plotted semi-logarithmically followed a straight line. The Z value of the curve was found to be 49.0.

A study was conducted to determine the effect of heat and storage on the loss of potency of small concentrations of K-penicillin in milk, 1% phosphate buffer (pH 6.0), and water. Also, a comparison was made of the heat stability of five different penicillins added to milk.

Penicillin concentrations of 0.13 to 1.07 I.U. per milliliter were added to water, buffer, and milk. Portions of each were subjected to temperatures of 143° F. for 30 minutes, 160° F. for 30 minutes, and 250° F. for 15 minutes. The unheated and heated lots were then stored at 34°-38° F. and assayed for penicillin every day for periods up to 7 days. Antibiotic activity was determined by the disk assay method.

The results revealed that penicillin in milk developed smaller zones of inhibition than did the same concentration in buffer or water.

Five different kinds of penicillin were found to vary in heat stability.

Upon heat treatment, penicillin was destroyed in an increasing order in milk, buffer, and water. On storage, the penicillin lost its potency at a faster rate in milk and water than in buffer. Also, the storage losses of the antibiotic were less in the milk samples heated at higher temperatures than in the raw samples or in the samples pasteurized at 143° F.

Within the limits studied, the concentration of the antibiotic and degree of inactivation by heating or storage exhibited no relationship.

Penicillin in milk was found to be relatively more heat stable, but less stable during storage than were streptomycin and aureomycin in previous studies *(15, 16)*.

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EVIDENCE OF MALTOL AND HYDROXYMETHYLFURFURAL IN EVAPORATED MILK AS SHOWN BY PAPER CHROMATOGRAPHY[1]

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Isolation and identification of specific heat-generated products has been an approach used by some workers in studying the effects of heat on milk *(12)*. Such an approach requires heating milk for prolonged periods at autoclaving temperatures (121° C., several hours) in order to produce sufficient yields of compounds for identification. In this manner, maltol *(10)*, hydroxymethylfurfural *(11)*, and other compounds have been identified. The question arises whether these compounds are produced in milk by normal heat-sterilizing treatment (116° C., 15 minutes). Paper chromatography has made possible an investigation of this point, and evidence has been secured that the two mentioned compounds may be present in representative retail samples of evaporated milk, as well as in more highly heated milks.

EXPERIMENTAL METHODS

The paper chromatographic separation of maltol and hydroxymethylfurfural was made with Whatman No. 1 filter paper with descending flow in a Fisher chromatographic apparatus and a 12 × 24 in. glass jar. To equilibrate the atmosphere before starting the solvent flow, a portion of the solvent or the unused phase in a two-phase system was placed in a beaker at the bottom of the jar. Filter paper strips 6 × 22.5 in. were used. The materials under study were spotted on the filter paper with a 0.01-ml. pipette. The papers were maintained at room temperature until the spot areas were completely dry before placing them in the jar. An equilibration period of 1 to 2 hours was allowed before the addition of the solvent. The solvent flow varied from 6 to 18 hours, depending upon mobility of the solvent. On removal of the papers from the jar, the solvent front was marked and the papers were air-dried at room temperature. Detection of the spot areas was accomplished by spraying the paper on both sides with the appropriate reagents. If heat was necessary to accomplish the color-producing reactions, the papers were placed in a thermostatically controlled laboratory oven.

The demonstration of furfuryl alcohol *(13)* in heated milk by paper chromatographic means was attempted. Despite considerable effort, an entirely suitable combination of chromatographic methods could not be developed. The

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compound was observed to volatilize from paper within a matter of several hours. The only spray reagent found that gave a sensitive color reaction (red-brown) with furfuryl alcohol was an acid solution of diphenylamine *(4)*.

Preparation of concentrated extract from heated milk. To insure adequate production of the compounds under study, highly heated milk was prepared by autoclaving sealed 14-oz. cans of fresh condensed skimmilk (approximately 29% total solids) obtained from the University Creamery. The heat treatment was for 2 hours at 120° C., after which the cans were cooled in cold water and then transferred to 4° C. storage until used. Direct ether extraction of the heated milk or serum expressed from the curd invariably resulted in troublesome emulsions. It was found that treatment of the heated milk with acetone eliminated these difficulties. For this treatment four 14-oz. cans of heated milk were mixed thoroughly with twice as much acetone by volume. The mixture was filtered by using suction, and the curd was again mixed with the same quantity of acetone. The mixture was again filtered, and the two filtrates were combined. Most of the acetone was removed by evaporation on a steam bath, and heating was discontinued when the temperature of the vapors reached 70° C. The remaining milk serum was cooled and filtered. The serum was adjusted to pH 7.2 with dilute sodium hydroxide and extracted for 40 hours with ethyl ether in a continuous extractor (Ace Glass Company). The ether extract was concentrated by removal of the solvent, and the remaining residue was used for spotting on the paper chromatograms. A concentrated extract was obtained from commercial brands of evaporated milk in the same manner except that no adjustment was made in the pH of the serum.

Chromatography of maltol and hydroxymethylfurfural. The following solvent systems and spray reagents were found applicable for the resolution and identification of maltol and hydroxymethylfurfural on paper chromatograms: *(a)* isoamyl alcohol and 5 *N* formic acid, equal parts by volume employed as by Buch *et al. (2)*, *(b)* isoamyl alcohol, concentrated NH_4OH, and water (30-15-5 by volume), Siegel and Schlogl *(14)*, and *(c)* ethyl acetate, pyridine, and water (5-2-7 by volume), McFarren *et al. (8)*.

Spray reagents employed in the detection of maltol were as follows: *(a)* equal parts by volume of 0.1 *N* $AgNO_3$ and 0.1 *N* NH_4OH, mixed just prior to use *(2)*; the sprayed chromatogram was dried at room temperature, and the orange color for maltol appeared within 15 minutes; *(b)* a 0.5% aqueous solution of ferric chloride *(5)*; maltol produced an immediate purple color.

Spray reagents used for detection of hydroxymethylfurfural were: *(a)* 2,4-dinitrophenylhydrazine (0.40 g.) dissolved in 100 ml. of 2 *N* HCl *(1)*; an immediate yellow spot was obtained; *(b)* benzidine (0.50 g.) dissolved in 20 ml. of glacial acetic acid and 80 ml. of ethyl alcohol *(7)*; as the sprayed chromatogram was dried at room temperature a faint light brown color was visible, which was intensified by heating at 110° C. for 10 minutes; *(c)* a mixture of equal quantities by volume of 1.0% alcoholic phloroglucinol and 20% aqueous trichloroacetic acid *(9)*; heating the papers at 110° C. for 15 minutes produced a reddish-brown spot with hydroxymethylfurfural; *(d)* orcinol (0.5 g.) and 15 g. of trichloro-

acetic acid added to 100 ml. of water-saturated butanol *(15)*; a green color was formed with hydroxymethylfurfural when the papers were heated at 110° C. for 10 minutes, and when observed under ultraviolet light the spot emitted a characteristic greenish-yellow fluorescence.

Identification of maltol and hydroxymethylfurfural in heated milk. Chromatograms were made containing authentic samples[3] of maltol, hydroxymethylfurfural, and the heated milk extract. The solvent system of isoamyl alcohol and formic acid was used with a 16-hour flow time. After being dried, one paper was sprayed with ferric chloride solution and the second with the orcinol reagent. When the chromatogram was sprayed with ferric chloride, a purple spot in the heated milk was congruent with maltol (R_f 0.76). The orcinol reagent showed a spot congruent with hydroxymethylfurfural (R_f 0.76). On other chromatograms, maltol was detected in the heated milk by using the silver nitrate reagent. By spraying with benzidine, 2,4-dinitrophenylhydrazine, and phloroglucinol reagents, hydroxymethylfurfural was detected in the heated milk extract. To provide additional information that maltol and hydroxymethylfurfural were being detected, other solvent systems were used. After the chromatograms were sprayed with the appropriate reagents, spots congruent with maltol and hydroxymethylfurfural were obtained with the heated milk sample in all solvent systems. The R_f values obtained with these solvent systems are presented in Table 1.

TABLE 1

Comparative R_f values for maltol and hydroxymethylfurfural with various solvent systems

Solvent	R_f value	
	Maltol	Hydroxymethylfurfural
Isoamyl alcohol—formic acid	0.76	0.76
Isoamyl alcohol—NH_4OH—water	0.23	0.64
Ethyl acetate—pyridine—water	0.79	0.88

With the development of satisfactory paper chromatographic procedures for the resolution and identification of maltol and hydroxymethylfurfural in highly heated milk, attention was given to evaporated milk. Four cans of a commercial product were purchased on the local market. The contents of the cans were combined and after treatment with acetone and extraction with ether, approximately 5 ml. of ether soluble material was obtained from this milk. This material was extracted three times with petroleum ether to remove the fatty material. The petroleum ether insoluble residue amounted to only a few drops; however, the quantity was sufficient for several chromatograms. When the irrigated chromatograms were sprayed with ferric chloride and orcinol reagents, spots were found congruent with those for maltol and hydroxymethylfurfural. A second brand of evaporated milk gave identical results.

Control experiments. The absence of maltol and hydroxymethylfurfural in unheated milk has been shown previously *(10, 11)*. However, the manipulative

[3] Hydroxymethylfurfural was obtained by synthesis *(11)* and maltol through courtesy of Cliffs-Dow Chemical Co., Marquette, Mich.

procedures for preparing heated milk extracts in this study revealed the need for parallel treatment of control samples. For this purpose the fresh condensed skimmilk, previously described, was used. The maximum heat treatment which this product had received was 65.6° C. for 20 minutes during forewarming. In preparing extracts from this milk, conditions identical to those used for the highly heated condensed skimmilk and evaporated milks were employed. The extracts, from several control experiments with four 14-oz. cans of the condensed skimmilk, were chromatographed on paper with the isoamyl alcohol–formic acid solvent system. The presence of hydroxymethylfurfural on these chromatograms was investigated with the benzidine and 2,4-dinitrophenylhydrazine spray reagents. Efforts to detect maltol were made with the ferric chloride reagent. No evidence of either maltol or hydroxymethylfurfural in extracts from the "unheated" product could be secured, whereas under comparable conditions both of these compounds were readily revealed in extracts from either the highly heated milk or the evaporated milks.

DISCUSSION

Since its development for the separation of amino acids *(3)*, paper chromatography has been used in the study of a wide variety of organic and inorganic compounds. The identification of compounds by the method is not infallible. However, if identical color reactions of the known and unknown can be obtained with at least two different spray reagents and corresponding R_f values obtained in two different solvent systems, the chances of error are very small.

Maltol apparently has not been resolved and identified previously by paper chromatography. Gottschalk and Partridge *(6)*, in studying the interaction of glucose and lysine by paper chromatography, obtained a spot congruent with hydroxymethylfurfural that gave the same color reaction when sprayed with naphthoresorcinol or aniline hydrogen phthalate reagents. They used a solvent system of butanol and acetic acid. In the present study three additional solvent systems were found applicable, as well as four other spray reagents. These same systems could also be used for the resolution of maltol. Of these solvent systems, the most distinct spots were obtained with the isoamyl alcohol and formic acid solvent. Ferric chloride had greater sensitivity for the detection of maltol than the silver nitrate spray reagent. For hydroxymethylfurfural the orcinol spray reagent had an advantage in that a greenish-yellow fluorescence was obtained under ultraviolet light even though the spot was not otherwise visible.

The most significant results obtained in this study concern evaporated milk. By the methods of paper chromatography developed for the separation and detection of maltol and hydroxymethylfurfural, these compounds were detected qualitatively in commercial evaporated milks. Although the amounts involved are extremely small, the need for quantitative methods of measurement for such compounds in milk is evident. Such methods would aid considerably in revealing the roles of heat and storage in formation of the compounds and in evaluating heat damage in sterilized milks.

SUMMARY

Paper chromatographic methods are presented for the resolution and identification of maltol and hydroxymethylfurfural. By using such methods these compounds were detected in highly heated milk (120° C., 2 hours) and in samples of evaporated milk produced and distributed under commercial conditions. Neither of these compounds could be detected in milk receiving limited (65.6° C., 20 minutes) heat treatment.

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THE EFFECT OF SYNTHALIN A ON BLOOD SUGAR IN DAIRY CATTLE[1]

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Histologically, three types of cells, alpha, beta, and delta, can be demonstrated in the pancreatic islets. The beta cells are well known as the site of insulin production. The alpha cells appear to be secretory in type *(10, 14)* but have not yet been definitely characterized as hormone-producing cells. There is some indirect evidence that glucagon is produced by these cells *(1, 6)*. Glucagon is the name which has been given to the hyperglycemic-glycogenolytic factor (HGF) which can be extracted from the pancreas.

It is well known that alloxan and other substances exhibit a selective destruction of the beta cells of the pancreas. This "tool" has been very useful in studies of pancreatic function. A search for chemicals with a similar effect upon the alpha cells has shown that certain guanidine compounds have a profound effect. Watanabe *(15)* in 1918 demonstrated that guanidine hydrochloride produced a marked hypoglycemia in rabbits. Frank *et al. (7, 8, 9)* synthesized several polymethylenediguanidines and studied their effects on blood sugar levels. Both synthalin A (decamethylenediguanidine) and synthalin B (dodecamethylenediguanidine) were claimed to be effective in producing hypoglycemia, with synthalin B being less toxic. Bischoff *et al. (2, 3)* demonstrated that the degree of hypoglycemic action and toxicity are concurrent; synthalin B was less potent, physiologically, than synthalin.

A destruction of the alpha cells, after the administration of synthalin, with the beta cells apparently unaffected, has been demonstrated histologically by Davis *(4)* and by Fodden *(5)*. Fodden and Read *(6)* have demonstrated that although pancreatic extracts of normal and alloxan-treated rabbits contain the HCF factor, extracts from synthalin-treated rabbits are devoid of HGF.

The characteristic blood sugar changes after injection of synthlin A are: first, a pronounced hyperglycemia, presumably due to hyperfunctioning of the alpha cells; second, a sharp drop to an extremely hypoglycemic level which is considered, at least by some workers *(6)*, to be due to exhaustion and destruction of the pancreatic alpha cells. The evidence that the above changes in blood glucose produced by synthalin are secondary to the selective destruction of the pancreatic alpha cells is indirect. The possibility that the effect is directly due to a toxic effect on the liver has not been ruled out.

The following is a report of investigations on the effect of synthalin A on blood glucose levels in dairy animals.

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[1] Delaware Agricultural Experiment Station Miscellaneous Paper No. 229.

EXPERIMENTAL PROCEDURE

Six bull calves (five Guernseys, one Holstein) were injected intramuscularly with an aqueous solution of synthalin A (decamethylenediguanidine) in dosages varying from 1.0 to 2.0 mg. per pound body weight. A mature Holstein cow (age 11 years, weight ca. 1,200 lb.) was injected intramuscularly with 1 mg. synthalin A per pound body weight. After a 3-week recovery period the same cow was injected with 1.4 mg. synthalin A per pound body weight.

Blood samples were taken from the jugular vein at the following times: hourly for first 4 hours, at 2-hour intervals for the following 8 hours, and at various additional times up to 48 hours post-injection. Oxalate was used as the anticoagulant. Blood glucose was determined by the Nelson modification *(11)* of the Somogyi-Shaffer-Hartman method *(12, 13)*.

RESULTS AND DISCUSSION

The first dosage level studied was 1 mg. of synthalin per pound body weight, given to a Guernsey bull calf weighing approximately 100 lb. The results are shown in Figure 1. There was a rise in blood glucose level for the first hour

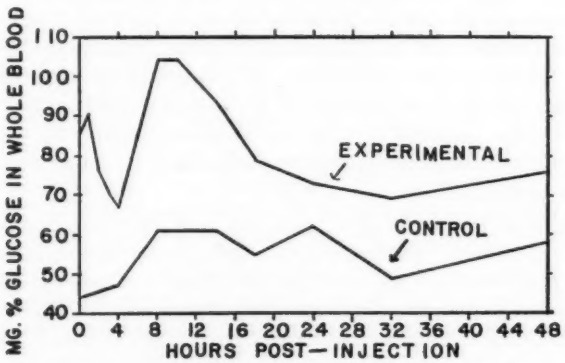

FIG. 1. Effect of 1 mg. synthalin A/lb body weight in a 100 lb. Guernsey calf.

followed by a drop during the next 3 hours, then a marked rise to above the initial value over the next 4 hours, followed by a gradual drop again. At no time did the experimental animal yield values below those of the control. The lowest point of the curve is well above levels exhibited by ketotic animals. The control was a Guernsey bull calf, pen-mate of the experimental calf. The comparison would be better if the initial blood glucose levels had been similar; however, the control served to show that the short rise followed by a drop in blood sugar levels seen in the first 4 hours after injection was probably due to the treatment. The authors are unable to explain the great difference in the initial blood glucose levels of these two calves.

A dosage of 1.3 mg. synthalin A per pound body weight was given to a 125-lb. Guernsey calf with the results indicated in Figure 2. The blood glucose levels

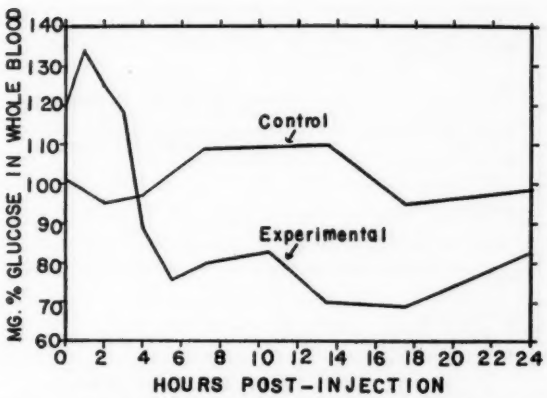

FIG. 2. Effect of 1.3 mg. synthalin A/lb body weight in a 125 lb. Guernsey calf.

of both the experimental and the control animal were well above those shown in Figure 1. The initial rise occurred during the same period as before but was more pronounced. There was a drop from the second to the sixth hour, and after 24 hours the level was still below that of the control and much below the initial value.

Dosage levels above 1.3 mg. per pound body weight were then given to four calves to determine if the presumed pancreatic alpha cell deficiency created by synthalin A could produce blood glucose levels as low as those seen in bovine ketosis. The results of these trials are shown in Figure 3. In each animal the injection of synthalin A resulted in an initial increase in blood glucose level followed by a very pronounced decrease. The minimum levels were in or below the range usually seen in ketotic cows.

In all young animals given 1.5 mg. per pound body weight, or less, uneventful recovery occurred. Dosages of over 1.5 mg. per pound body weight have resulted in convulsions and death even though in one animal the blood glucose level was as high at the time of death as levels exhibited by many normal cows. It seems apparent that in this case death did not result from hypoglycemia per se but rather from other toxic effects of the drug. Numerous investigators have reported toxic effects on the liver and kidneys when synthalin A has been administered.

The adult bovine has a characteristically low blood glucose level. The end-products of carbohydrate digestion are predominantly short chain fatty acids. Apparently very little glucose as such, is absorbed; therefore metabolic factors which affect glucose utilization may not have the same importance in the mature bovine as they have in the young calf. For this reason it was considered necessary to investigate the effect of synthalin A in a mature cow. The results of dosage levels of 1.0 and 1.4 mg. per pound body weight given 3 weeks apart are shown in Figure 4. The 1.0 mg. dosage level did not produce a hypoglycemia but did produce a marked diuresis and lethargy. Marked improvement was shown on

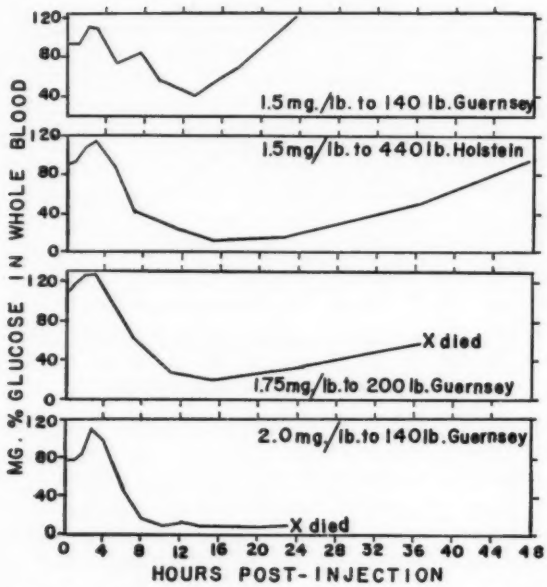
MG. % GLUCOSE IN WHOLE BLOOD
1.5 mg./lb. to 140 lb. Guernsey
1.5 mg./lb. to 440 lb. Holstein
X died
1.75 mg./lb. to 200 lb. Guernsey
2.0 mg./lb. to 140 lb. Guernsey
X died
HOURS POST-INJECTION

FIG. 3. Effect of lethal and near lethal doses of synthalin A upon blood glucose in dairy calves.

the third day post-injection followed by apparently complete recovery within a week. Administration of 1.4 mg. per pound body weight produced a very pronounced initial hyperglycemia followed by a hypoglycemia. Twelve hours post-injection the cow appeared dull, at 24 hours she would not attempt to stand, and death occurred at 34 hours. Lower blood glucose levels than she exhibited are often seen in ketosis; therefore, toxic effects of synthalin A on other organs or metabolic pathways were probably responsible for the death, rather than the low glucose level per se. Even though these data are from only one cow, they

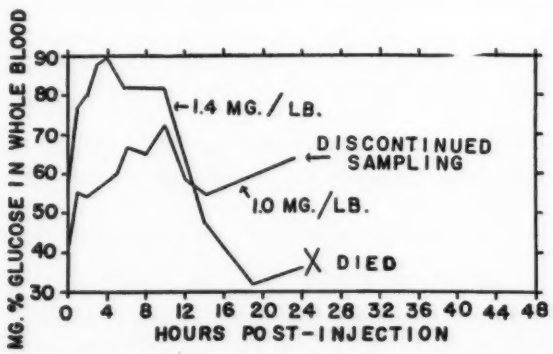

FIG. 4. Effect of synthalin A upon the blood glucose level of a 1,200 lb., 11-year-old Holstein cow.

show that the blood sugar homeostasis in the mature bovine is markedly upset by synthalin A. Sacrificing more cows to prove this point was considered not necessary.

It is apparent that the blood glucose level in the mature, as well as the immature, bovine is greatly affected by low levels of synthalin A. The degeneration of the pancreatic alpha cells shown when synthalin A was given to rabbits by Davis *(4)* and Fodden and Read *(5, 6)*, the complete lack of HGF in the pancreases of rabbits treated with synthalin A reported by Fodden and Read *(6)*, and the effect of synthalin A on the bovine blood glucose level reported in this paper suggest the possibility that the bovine is dependent upon the secretion of pancreatic alpha cells for glucose homeostasis. This evidence, however, is indirect, and the possibility exists that the observed effect upon blood glucose level is due to a toxic effect on other organs or metabolic pathways. Synthalin A does have a toxic effect on the liver and kidneys *(4)*.

SUMMARY

The effect of synthalin A (decamethylenediguanidine) upon blood glucose level in the bovine has been reported from studies with six dairy calves and a mature cow. In calves, dosages of 1.0 mg. per pound body weight given intramuscularly had little effect. A dosage of 1.3 mg. per pound resulted in an initial hyperglycemia followed by a marked hypoglycemia, but levels as low as those reported in cows with ketosis were not approached. At 1.5 mg. per pound dosages, or above, blood levels of glucose were lowered to less than 40 mg. % (some were near 10 mg. %). A dosage of 1.0 mg. per pound in the mature cow had an apparent hyperglycemic effect, but no hypoglycemia resulted. When 1.4 mg. per pound was injected, a pronounced initial hyperglycemia was followed by a marked hypoglycemia.

The blood glucose levels of the mature and immature bovine are drastically affected by synthalin A.

ACKNOWLEDGMENTS

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EFFECTS OF DIETARY ARSANILIC ACID ON THE GROWTH AND WELL-BEING OF YOUNG DAIRY CALVES[1]

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Certain organic arsenicals have been reported to be effective in stimulating the growth of chickens, turkeys, and swine *(1, 4, 6, 9)*. Frost *(4)* presumes that "one action of the phenylarsonic acids is similar to that of antibiotics—in some way altering bacterial metabolism in the tract to favor improved nutrition of the animal."

The effects of feeding arsenicals to dairy calves were determined by Graf and Holdaway *(5)*, who fed *p*-aminophenylarsonic acid at three levels, 60, 120, and 240 g., incorporated in each ton of milk replacement or calf starter. They reported a lower incidence of scours and more rapid gains during the first 4 weeks after birth. The calves fed levels of 60 or 120 g. per ton of feed made slightly greater gains than those fed the 240-g. level. Owen *et al. (8)* reported greater weight gains for calves fed 3-nitro-4-hydroxyphenyl arsonic acid at the rate of 57 p.p.m. of total dry matter in ration than for controls or for calves fed terramycin. However, calves fed the arsonic acid made gains that were similar to gains made by calves fed bacitracin and less than gains made by calves fed terramycin. Dewey *(3)* supplemented the diets of calves between 5 and 90 days of age with 20 mg. of sodium arsanilate daily per 100 lb. body weight. No difference in growth rate was observed between the supplemented and control calves. Also, supplementation of the diet of 4-month-old calves on pasture with 40 mg. of sodium arsanilate daily per 100 lb. body weight did not produce any increase in growth rate. However, with five 4-month-old calves that had been retarded in growth for reasons other than lack of feed, significant increases in growth rate resulted when the calves were supplemented with 15 mg. of sodium arsanilate daily per 100 lb. body weight.

The experiment reported herein was undertaken to provide additional information on the feeding of arsenicals to calves.

EXPERIMENTAL PROCEDURE

Eighteen female calves (four Ayrshires, six Holsteins, and eight Jerseys) were paired by breed and age. From birth to 23 weeks of age, 50 mg. of *p*-aminophenylarsonic acid[2] (arsanilic acid) was given once daily by capsule to one member selected at random from each pair, the other calf of each pair serving as the control. All calves received their mothers' colostrum the first 3 days.

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[1] Contribution No. 241, Department of Dairy Husbandry, and No. 23, Statistical Laboratory, Kansas Agricultural Experiment Station, Manhattan.

[2] Through the courtesy of D. V. Frost, Abbott Laboratories, North Chicago, Ill.

TABLE 1
Effect of arsanilic acid on the weights of calves at 4-week intervals

Supplement	No. of calves	Weight in per cent of birth weight at:					
		4 wk.	8 wk.	12 wk.	16 wk.	20 wk.	23 wk.
Arsanilic acid[a]	9	118	160	204	262	311	355
None (Control)	9	124	168	218	266	311	358

[a] 50 mg. daily by capsule.

Beginning with the fourth day they were fed whole milk at approximately 10% of body weight daily for the next 4 weeks. After the fourth week, the rate of milk feeding was gradually reduced until a total of 350 lb. had been consumed. The calves usually were weaned from milk between 6 and 8 weeks of age. Alfalfa hay and a calf starter were fed ad libitum beginning at 1 week of age. At the 17th week of age, the calves were changed from the starter to a calf grower. Consumption of starter and grower was limited to 5 lb. for Holsteins and Ayrshires and 4 lb. for Jerseys. The calves were weighed at weekly intervals. Details on the composition of the feeds and on the method of feeding and management were described in a previous paper *(2)*.

RESULTS

The effect of arsanilic acid on growth is shown in Table 1. The calves varied in size owing to differences in breed, sex, and birth weight. Therefore, all weight data were converted to a comparable basis by converting the weekly body weights of each calf into a percentage of original birth weight, the original birth weight being considered 100 for each calf regardless of size. The growth data in Table 1 are presented by 4-week intervals to save space. There were no statistically significant differences in size between control calves and those fed arsanilic acid at any time during the 23-week period of study. This conclusion is based on 23 *t*-tests applied to weekly differences between the size of calves fed arsanilic acid and their paired controls.

The feed intakes were studied from birth to 16 weeks, inclusive, as this period was considered to be of sufficient duration to evaluate the effect of arsanilic acid on feed consumption. An analysis of variance was made of the body weight gain and feed efficiency for this period (Table 2). The differences between control calves and those fed arsanilic acid were small and not statistically significant.

TABLE 2
Effect of arsanilic acid on body weight gain and efficiency of gain of calves from birth to 16 weeks of age[a]

Supplement	No. of calves	Birth weight	Gain in wt.	TDN/lb gain[b]	Digestible protein/lb gain[b]
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
Arsanilic acid[c]	9	76	119	2.91	0.66
None (Control)	9	73	114	3.04	0.69

[a] None of these differences was statistically significant.
[b] Calculated from Morrison's *(7)* tables.
[c] 50 mg. daily by capsule.

Study of the effects of the experimental treatments on calf health showed that the nine control calves had a total of 16 digestive and respiratory infections, five slight, two medium, and nine severe. The nine calves receiving arsanilic acid had 15 infections, five slight, one medium, and nine severe. Thus, there was no apparent difference between control calves and those fed arsanilic acid in incidence of disease. Also, there was no apparent difference in physical condition between the two groups of calves. In the environment of this experiment, neither beneficial nor deleterious effects resulted from feeding 50 mg. of arsanilic acid daily per calf.

SUMMARY

This experiment was initiated to study the effects of arsanilic acid on the growth and well-being of young dairy calves. Eighteen calves were divided into two groups, balanced for age and breed. Calves in one group received 50 mg. of arsanilic acid daily per calf, and those in the control group received no arsanilic acid. All calves were started on experiment at birth and continued until 23 weeks of age.

There was no statistically significant difference between the two groups in regard to rate of weight gain or feed efficiency. Also, there was no apparent difference between the two groups in incidence of disease or in physical appearance.

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DRIED WHEY AND LACTOSE AS SUPPLEMENTS TO A VEGETABLE MILK REPLACER[1]

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The use of milk replacers for calf feeding is increasing in importance because more whole milk is being marketed. This practice results in less skimmilk being available for calf feeding. If low cost, nutritionally adequate milk replacers were available, the dairyman would be able to raise calves which now have to be sold.

The formulation of the first milk replacer is credited to Liebig by Kellner *(6)*. Since then, numerous formulas for milk-replacer feeds and methods of feeding have been proposed. According to Carpenter *(1)*, soybean oil meal is an excellent source of vegetable protein. However, Shoptaw *(14)*, using a soybean milk, and Wallace *et al. (18)*, using a milk replacement, reported that the soybean flour caused diarrhea in calves. Satisfactory results with calf starters containing 16 to 18% of a processed soybean oil meal were reported by Norton and Eaton *(11)*. They fed 350 lb. of whole milk per calf. Noller and Huffman *(10)* reported the successful use of a special processed soybean flour as the principal source of protein in calf rations. The total amount of whole milk which was fed varied from 59 to 89 lb. per calf. Later, Stein *et al. (16)* and Stein and Knodt *(15)* reported the successful use of soybean flour in milk-replacer rations.

Dried whey has been used in calf rations with varied results *(5, 17, 18, 19)*. Lactose at the 20, 25, and 30% levels caused diarrhea *(12)* but 5% and 10% lactose had a favorable effect *(4)*. The physiological effects of lactose were reviewed by Fischer and Sutton *(3)* and Duncan *(2)*.

The purpose of this paper is to report the results of an investigation concerning the nutritive value of dried whey and lactose as supplements to an all-vegetable milk-replacer ration.

EXPERIMENTAL PROCEDURE

Seventeen male and four female Holstein calves, obtained from the University herd and local dairymen, were divided into three lots of seven calves each. The groups were adjusted on a comparable initial weight basis. One group received the basal all-vegetable replacer ration (replacer 1) and served as the control group. The second group received 5% dried whey as a supplement to

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[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1832.

[2] This article is part of a dissertation presented to the faculty of the School for Advanced Graduate Studies of Michigan State University in partial fulfillment of requirements for the degree of Doctor of Philosophy.

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TABLE 1
Daily feeding schelule

Age	Whole milk	Replacer fed as gruel	Replacer fed dry
(days)	*(lb.)*	*(lb.)*	*(lb.)*
0-2	with cow	—	—
3	usually fasted	—	—
4-6	6	—	—
7-10	4	0.5	ad libitum
11-21	2	0.5	ad libitum
22-60	—	0.5	ad libitum

the all-vegetable ration (replacer 2). The third group received 3.5% lactose as a supplement (replacer 3).

A uniform procedure for handling the young calves was followed throughout. The calves remained with their dams for the first 48 hours after birth. They were then weighed, placed in individual pens, bedded with wood shavings, and usually fasted for the next 24 hours. Whole milk was fed on the fourth day of life and continued with decreasing amounts through the 21st day. The maximum amount of whole milk fed was 56 lb. Any calf that was apparently sick or abnormal during the first few days on experiment was removed, and another calf was substituted in its place. On the seventh day, 0.5 lb. of a milk replacer was fed as a gruel and the dry milk replacer was made available for ad libitum feeding. The amount of water fed daily was calculated to supply a total liquid intake of 1 lb. per 10 lb. of body weight. No hay was fed at any time. The experiment was terminated when the calves reached 60 days of age. The daily feeding schedule is presented in Table 1.

TABLE 2
Composition of the milk replacers

Ingredients	Milk replacer		
	1	2	3
	(%)	*(%)*	*(%)*
Fine ground corn	51.6	47.1	47.1
Soybean flour (52.4%)	33.0	32.5	34.0
Distillers dried solubles	10.0	10.0	10.0
Dried whey	—	5.0	—
Lactose (c.p.)	—	—	3.5
Steamed bonemeal	1.5	1.5	1.5
Calcium carbonate	0.5	0.5	0.5
Salt	0.5	0.5	0.5
Aurofac[a]	2.8	2.8	2.8
Vitamin and trace mineral mixture[b]	0.1	0.1	0.1
Total protein	26.94	26.69	25.37

[a] Contained 1.8 g. of aureomycin and 1.8 mg. of B_{12} activity per pound.
[b] Mixture contained:

Vitamin A concentrate 20,000 U.S.P. units/g.	20 g.
Irradiated yeast, 9,000 I.U. vitamin D/g.	5 g.
Cobaltous sulfate ($CoSO_4 \cdot 7H_2O$)	3 g.
Cupric sulfate ($CuSO_4 \cdot 5H_2O$)	2 g.
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	11 g.

The formulas for the three milk-replacer rations are presented in Table 2. The protein content of each ration was made approximately the same by varying the amounts of corn and soybean flour added to each ration. An aureomycin-containing feed supplement (Aurofac) was added to each replacer at a level calculated to supply 50 mg. of aureomycin and 50 γ of vitamin B_{12} activity per pound of feed.

The calves were weighed at the beginning of the experiment, at approximately the same hour on two consecutive days each week during the experiment, and at the completion of the experiment.

RESULTS AND DISCUSSION

Feed consumption among the three groups of calves was similar. The average amount of whole milk consumed during the experimental period varied from 50.7 to 54.9 lb. per group (Table 3). The variation among the groups was attributable to a few calves that refused one or more feeds immediately after

TABLE 3

Comparison of the gains in body weight and feed consumption data obtained from the experimental calves

	Replacer		
	1	2	3
No. of calves	7	7	7
Weight			
Av. initial *(lb.)*	91.4	93.9	93.6
Av. daily gain *(lb.)*	0.87	0.92	0.87
and S. E. of mean	0.08	0.05	0.07
Av. total feed intake per calf			
Whole milk *(lb.)*	54.9	51.9	50.7
Milk replacer *(lb.)*	108.2	117.5	112.9
Replacer per lb. of gain *(lb.)*	2.21	2.26	2.37

the milk replacer was added to the liquid. In general, little or no difficulty was encountered when the gruels were fed. During the first 20 to 30 days of age, the individual calves consumed 1 lb. or less per day of the replacer ration, but after a time consumption increased to 4 to 5 lb. daily. The consumption of the replacer ration varied more within groups than between groups.

Statistical analyses of the growth data indicate no significant differences in the average daily gain among the three groups of calves (Table 3). However, the calves which received the dried whey (replacer 2) gained more uniformly in body weight. The similarity in weight gains among the groups is shown graphically in Figure 1. The growth patterns also indicate a period of little or no gain in body weight during the first 20 to 30 days of age, which is referred to as the "critical period." After this period, the gain in body weight of the calves was approximately equal to the USDA standard for Holstein calves *(8)*. This standard was established with calves that received whole milk up to 4 weeks

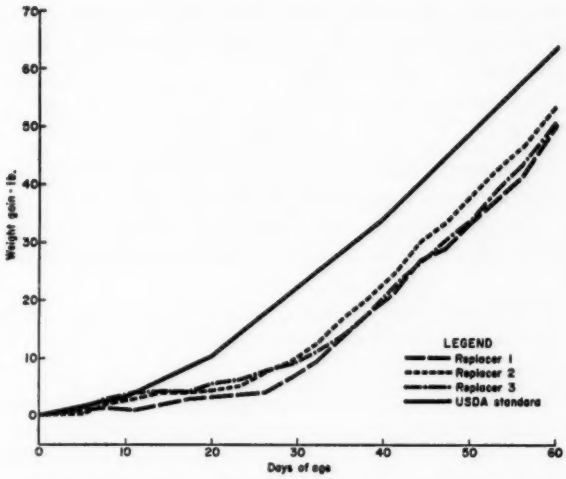

FIG. 1. Comparison of the growth curves obtained for the experimental calves with that for Holstein calves used to determine the USDA standard.

of age and then skimmilk up to 6 months of age. In contrast, the total amount of whole milk received by the calves in this experiment averaged 52.5 lb. per calf. The growth patterns for the experimental calves indicate that the milk replacers were inadequate for the calves during the critical period, but subsequently the milk replacers were approximately equal in value to skimmilk. Stein *et al. (16)* also observed a decrease in weight gains when whole milk was replaced by milk replacers at 10 days of age.

The condition of the feces improved simultaneously with the increase in feed consumption and weight gains. After the critical period the feces resembled those of the adult animal in odor and appearance. The change in fecal odor may be attributable to the improved utilization of protein with a consequent decrease in putrefaction in the large intestine. According to Schmidt *(13)*, putrefaction of protein occurs exclusively in the large intestine. The improved utilization of protein after the critical period may also coincide with initiation of rumen function. Kesler *et al. (7)* concluded from their rumen study with calves that rumen function begins between 32 and 42 days of age. This may vary with the type of ration and the criterion of response, since McMeekan *(9)* stated that calves on pasture began ruminating as early as 7 days of age and rarely later than 3 weeks.

Calves fed replacer 2, which contained 5% dried whey, were superior to the other calves in respect to fecal condition, alertness, and general appearance. The feces of these calves were most costive, suggesting a favorable effect which may be mediated through the intestinal flora. The addition of 3.5% lactose to the basal ration had no noticeable beneficial effect on the growth, feed consumption, or condition of the calves. Diarrhea was not a problem in this experiment, owing

to the low level of lactose ingested. The basal ration may be a critical factor in lactose evaluation, since Flipse *et al. (4)* reported favorable effects with 5% and 10% lactose in a synthetic milk ration.

SUMMARY

Twenty-one Holstein calves were divided into three groups and placed on three milk-replacer rations; basal, basal plus 5% dried whey, and basal plus 3.5% lactose. The individual calves in the three groups consumed an average total of 54.9, 51.9, and 50.7 lb. of whole milk, respectively, during the experimental period.

The average daily gains of the calves on the basal, whey, and lactose rations were 0.87 ± 0.08, 0.92 ± 0.05, and 0.87 ± 0.07 lb., respectively. The differences were not statistically significant.

Growth patterns indicated a critical period in the life of the young calf from birth to approximately 25 days of age. The end of the critical period was characterized by an increase in feed consumption, increased body weight, improved appearance of the calf, and a change in odor and appearance of the feces.

Calves receiving the whey ration had more costive feces and smoother haircoat and were more alert. Lactose appeared to have no benefit under the conditions of this experiment.

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COMPARISON OF SILAGES MADE FROM FIELD CORN (OHIO M15) AND SILAGE CORN (EUREKA) FOR MILK PRODUCTION[1]

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Previous papers *(6, 7)* have shown that the total digestible nutrients (TDN) in corn silage are superior for milk production to those found in hay. Since the chemical nature of the compound(s) which increases the net energy value of some feeds is unknown, it has been referred to as the unidentified lactation factor(s). Dunn *et al. (2)* recently demonstrated that the corn grain present in corn silage increased the milk-producing value of the silage. This factor was also present in corn-ear silage *(3)*.

The nutritive value of immature corn silages, however, has not been determined except in rations that already contained ample amounts of grain. The early literature dealing with the relative value of corn harvested at different stages of maturity was reviewed by Nevens *(10)*. Nevens pointed out that the conclusions reached by the early investigators concerning the suitability of a particular variety of corn for silage apparently were based on *(a)* yields of fresh matter or *(b)* yields of dry matter. Such conclusions are likely to be misleading. Hayden and Perkins *(4)* used milking cows to determine the feeding value of early and late maturing varieties of corn for silage. The earlier maturing corn was harvested 21 days sooner and contained more corn grain, but the silage made from later maturing corn seemed to be more efficient for milk and fat production. The cows gained more body weight, however, on the earlier maturing corn silage. The difference in weight may have been due to a bigger TDN intake. White and Johnson *(15)* fed early, medium, and late maturing corn silage, on an equal dry matter basis, in rations that already contained ample amounts of grain and found no significant difference in milk production. Nevens *(10)* compared a mature ear-producing variety of corn silage with two immature varieties. All of the rations contained supplemental grain. The immature varieties were found to have a lower value for milk production and also were less appetizing than the mature variety. The lower productive value of the immature varieties may have been due to the consumption of less dry matter and TDN.

In view of the above reports in the literature concerning the feeding value of immature corn silage, experiments were conducted with dairy cattle to compare the amounts of milk produced when an immature silage (Eureka) and a grain variety of corn silage (Ohio M15) were fed, both with and without supplemental grain.

EXPERIMENTAL PROCEDURE

The early maturing corn (Ohio M15) and the late maturing corn (Eureka) were grown in strips next to each other in the same field and were harvested at

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about the same time. The early maturing corn was in the dent stage and the late variety was in the milk stage. The two corn crops were chopped into separate silos.

Just before the two crops were harvested for silage, randomized samples of the green corn were collected from the field. From these samples the following data were calculated: yields per acre, 10.3 and 16.8 tons; dry matter, 6,236 and 7,042 lb.; corn grain per acre (14% moisture basis), 61.7 and 6.8 bu.; and the amount of corn silage required to supply 1 lb. of 14% moisture corn grain, 6.0 and 88.6 lb., respectively. The relative stage of maturity of each silage is indicated by the moisture content as well as by the stage of development of the kernels. The mature silage (Ohio M15) contained 69.8 compared with 79.1% moisture in the immature silage (Eureka), but the Eureka silage yielded 806 lb. more dry matter per acre than the Ohio M15.

The description and chemical composition of the feeds used in the experiment are presented in Table 1. Both silages were of excellent quality. The hay portion of the rations consisted of either first cutting, U. S. grade No. 3 alfalfa-brome or second cutting, U. S. grade No. 1 alfalfa. The grain mixture consisted of three parts of corn and one part of solvent-extracted soybean oil meal, except when otherwise indicated.

TABLE 1

Description of feeds used in the experiment

Feed No.	Feedstuff	Dry matter	Ash	Protein	Ether ext.	Crude fiber	NFE	DCP[a]	TDN
		(%)							
1[b]	Corn silage (Eureka)	20.9	0.91	1.76 53	0.64 73	6.6 66	11.0 62	0.93	13.1
2	Corn silage (Ohio M15)	30.2	1.66	2.76 53	1.04 74	6.1 66	18.7 69	1.46	20.1
3[c]	Alfalfa-brome (1st cut., U.S. No. 3)	87.9	5.3	14.0 62	1.58 35	35.7 56	31.3 64	8.7	49.9
4[d]	Alfalfa (2nd cut., U.S. No. 1)	89.7	6.1	16.0 74	2.40 33	27.5 43	37.7 72	11.8	52.6
5	Corn (U.S. No. 2)	86.8	3.1	8.6 77	4.40 90	2.5 57	68.2 93	6.6	80.4
6	Soybean oil meal	90.6	5.6	46.3 92	3.70 47	4.6 87	30.4 94	42.6	79.1

[a] Digestible crude protein.

[b] First line represents chemical composition and second line represents Morrison's *(9)* coefficients of digestibility, except for the alfalfa-brome hay. These values were determined experimentally.

[c] Used in Trials 1, 2, 8, 9, 10 (Table 2).

[d] Used in Trials 3, 4, 5, 6, 7 (Table 2), and for all trials in Table 3.

The coefficients of digestibility were determined for the alfalfa-brome hay, but the coefficients reported by Morrison *(9)* were used for the other feeds. The coefficients used for the Eureka silage were Morrison's values for immature dent corn silage (Southern type), and the values for well-matured dent corn (all analyses) were used for the Ohio M15 silage.

Thirteen Holstein and two Jersey cows were used in 22 trials; 12 trials with, and 10 trials without, a grain supplement. The periods were 18 days in length, except the first period, which was reduced to 12 days in order to minimize, as much as possible, the effect of the decline in milk production due to the advance in lactation.

The milk was weighed at each milking. Fat was determined on each 3-day composite sample. The amount of milk produced is expressed on the fat-corrected (FCM) basis. The cows were weighed once in every 3-day period.

RESULTS

The results of the comparison of the mature and immature silages fed without supplemental grain are presented in Table 2. Eureka silage was fed first in Trials 1 to 7, inclusive, followed by the Ohio M15. In Trials 8, 9, and 10, Ohio M15 was fed first, followed by Eureka and then Ohio M15 again.

TABLE 2

Comparative feeding value of Eureka (a) and Ohio M15 (b) corn silages for milk production (fed without supplemental grain)

Trial No.	In milk	Body wt.	Test	FCM	Intake of			TDN		Corn[a]
					Hay	Silage	DM	Rec.	Req.	
	(days)	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
1 a	78	1,040	3.3	22.2	14.6	59.3	25.2	15.1	15.3	—
b	90	1,015	3.1	22.9	15.0	38.0	24.7	15.1	15.4	5.1
2 a	93	733	4.4	9.5	11.1	40.0	18.1	10.8	9.4	—
b	105	719	4.4	10.1	11.3	25.0	17.5	10.7	9.6	3.3
3 a	250	1,090	3.3	15.0	15.0	59.6	25.9	15.7	13.3	—
b	262	1,088	3.0	13.9	15.0	40.0	25.5	15.9	13.0	5.3
4 a	201	1,069	3.2	18.3	15.0	85.6	31.4	19.2	14.3	—
b	213	1,076	3.1	18.5	15.0	60.0	31.6	19.9	14.4	8.0
5 a	226	1,131	3.8	19.6	15.0	70.4	28.2	17.2	14.5	—
b	238	1,120	3.7	19.5	15.0	46.0	27.4	17.1	14.4	6.1
6 a	341	1,030	5.1	10.2	15.0	54.5	24.9	15.1	12.7	—
b	353	991	4.8	10.9	15.0	36.0	24.3	15.1	11.4	4.8
7 a	189	1,062	3.8	14.0	15.0	77.6	29.7	18.1	12.9	—
b	201	1,085	3.8	13.5	15.0	40.9	25.8	16.1	12.8	5.5
8 b	218	1,071	3.4	18.2	15.0	49.2	28.1	17.4	14.3	6.6
a	230	1,097	3.3	17.3	14.7	75.3	28.7	17.3	14.1	—
b	248	1,084	3.6	18.5	14.9	45.1	26.7	16.5	14.4	6.0
9 b	85	1,069	2.8	22.3	15.0	31.5	22.7	13.8	15.6	4.2
a	97	1,082	3.3	22.3	15.0	75.4	29.0	17.4	15.6	—
b	115	1,072	3.4	25.1	15.0	45.2	26.8	16.6	16.5	6.0
10 b	109	1,093	2.9	18.8	15.0	47.6	27.6	17.1	14.6	6.3
a	121	1,083	3.2	18.3	14.8	73.0	28.3	17.0	14.4	—
b	139	1,068	3.2	19.8	14.8	43.1	26.0	16.1	14.7	5.7
Average										
a	—	1,042	3.7	16.7	14.5	67.1	26.9	16.3	13.7	—
b	—	1,032	3.5	17.1	14.6	41.7	25.6	15.9	13.6	5.6

[a] Estimated amount of corn grain fed in the Ohio M15 silage.

There was a slight increase in the production of FCM in Trials 1, 2, 4, and 6 when the cows were changed to Ohio M15, and about the same amounts of TDN and dry matter were consumed. There was a decline in Trials 3 and 7 and a slight decline in Trial 5. The decline in Trial 7 was probably due to a lower consumption of TDN and dry matter (2.0 and 3.9 lb. less) because the cow's appetite for the Ohio M15 silage was poor. When the Eureka silage followed the Ohio M15 in Trials 8 and 10, the amount of FCM decreased, but production increased when the cows were changed back to the Ohio M15. No change in FCM occurred in Trial 9 when the cow was changed to the Eureka silage, but the amount of FCM increased 2.8 lb. per day when the cow was changed back to the mature silage. This cow consumed 22.7 lb. of dry matter per day during the initial Ohio M15 period, 29.0 lb. during the Eureka period, and 26.8 lb. when changed back to the mature silage.

The data showing the relative productive value of Eureka and Ohio M15 silages are summarized at the bottom of Table 2. In Trials 8 to 10, inclusive, the average value for each two Ohio M15 periods, rather than individual values, was used to determine the average value for all trials. These data do not indicate any marked differences in body weights or in the amount of FCM produced that can be attributed to the superiority of the feeding value of mature silage over an immature silage. The cows consumed 1.3 lb. more dry matter and 0.4 lb. more TDN per day during the Eureka silage periods than during the Ohio M15 periods. The cows also received from 3.3 to 8.0 lb. (av. 5.6) of corn grain (14% moisture basis) from the silage during the Ohio M15 periods. The data for the amount of corn in the Eureka silage are omitted, but less than 0.8 lb. of corn per day was received by the cows during these periods.

The results showing the comparative feeding value of Eureka and Ohio M15 corn silage when a grain supplement was included in the ration are shown in Table 3. In Trials 1 to 5, inclusive, Eureka silage was fed first, followed by Ohio M15 and then Eureka, whereas in Trials 6 and 7, Ohio M15 was fed first, followed by Eureka and then Ohio M15. In Trials 1 to 4, inclusive, 2 lb. of soybean oil meal was fed with both silages, but in all the other trials a mixture of 25% soybean oil meal and 75% corn was included in both silage rations. No hay was fed in Trial 8.

In Trials 1, 2, and 4, the amount of TDN received was less than the required amount, according to the National Research Council's recommendations *(8)*. In all other trials, the amount of TDN was equal to or in excess of the requirements, especially in Trials 6 and 12.

The data showing the feeding value of the two silages when either soybean oil meal or the grain mixture was fed as a supplement to the silage rations are shown in Table 3. In the first five trials, Eureka silage was fed first, followed by Ohio M15 and then Eureka. In Trials 6 and 7, Ohio M15 was fed first, followed by Eureka and then Ohio M15. In Trials 1, 2, and 4, the cows received less TDN than the recommended requirement, but the amount of TDN fed in Trials 3, 5, 6, and 7 was equal to or exceeded the requirements, especially in Trial 6. The data for Trials 1 to 7 also are summarized in Table 3. These data

TABLE 3

Comparative feeding value of Eureka (a) and Ohio M15 (b) corn silages for milk production (fed with supplemental grain)

Trial No.	In milk	Body wt.	Test	FCM	Intake of				TDN		Corn[a]
					Hay	Silage	Grain	DM	Rec.	Req.	
	(days)	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
1 a	71	1,208	3.2	30.2	15.0	70.0	2[b]	29.9	18.7	18.9	—
b	83	1,190	3.1	31.4	15.0	46.0	2	29.2	18.7	19.2	6.1
a	101	1,244	2.8	33.0	15.0	70.0	2	29.9	18.7	20.0	—
2 a	35	1,049	2.8	34.6	15.0	60.0	2	27.8	17.4	19.4	—
b	47	1,038	2.9	34.3	15.0	40.0	2	27.4	17.5	19.2	5.3
a	65	1,072	3.0	34.8	15.0	70.0	2	29.9	18.7	19.6	—
3 a	75	1,206	3.2	32.1	15.0	99.4	2	36.0	22.6	19.5	—
b	87	1,197	3.2	34.9	15.0	66.0	2	35.2	22.7	20.3	8.8
a	105	1,199	3.2	34.0	15.0	70.0	2	29.9	18.7	20.1	—
4 a	48	1,220	3.4	35.9	15.0	73.2	2	30.6	19.1	20.8	—
b	60	1,172	3.1	36.5	15.0	49.0	2	30.1	19.3	20.7	6.5
a	78	1,192	3.3	35.4	15.0	70.0	2	29.9	18.7	20.5	—
5 a	43	1,348	3.4	55.7	15.0	80.0	12	40.6	28.1	28.0	—
b	55	1,360	3.4	57.4	15.0	53.0	12	39.9	28.2	28.6	7.1
a	73	1,379	3.6	56.9	15.0	70.0	12	38.5	26.8	28.6	—
6 b	203	1,338	3.1	42.7	15.0	47.3	12	38.2	27.0	23.7	6.3
a	215	1,329	3.2	41.3	14.4	76.9	12	39.4	27.3	23.2	—
b	233	1,328	3.5	43.8	14.3	46.0	12	37.1	26.4	24.0	6.1
7 b	189	1,202	3.8	24.9	10.0	49.0	4	27.2	18.3	17.2	6.5
a	201	1,216	4.0	22.7	10.0	77.7	4	28.7	18.7	16.6	—
b	219	1,227	4.0	19.9	9.9	45.5	4	26.1	17.6	15.7	6.1
Average											
a	—	1,229	3.3	36.5	14.2	74.4	5.1	32.8	21.4	21.1	—
b	—	1,215	3.3	37.2	14.2	49.7	5.1	32.3	21.6	21.2	6.6
8 a	33	1,165	3.5	61.2	—	90.0	20	36.2	27.9	28.6	—
b	45	1,158	3.2	57.2	—	60.0	20	35.5	28.1	27.2	8.0
9 a	229	1,133	3.4	23.1	9.7	70.9	4	27.0	17.7	16.2	—
b	241	1,112	3.4	24.2	9.5	43.2	4	25.0	16.9	16.4	5.8
10 a	132	1,175	3.3	26.6	10.0	74.7	6	29.8	19.9	17.6	—
b	144	1,163	3.4	26.7	10.0	39.9	6	26.2	18.1	17.5	5.3
11 b	265	1,116	3.3	22.5	9.9	47.9	4	26.8	18.1	15.9	6.4
a	277	1,149	3.3	18.2	9.4	62.6	4	25.0	16.4	14.7	—
12 b	257	1,314	3.1	40.5	12.2	50.0	12	36.5	26.1	22.9	6.7
a	269	1,347	3.1	35.1	13.9	69.8	12	37.5	26.2	23.1	—
Average											
a	—	1,194	3.3	32.8	10.8	73.6	9.2	31.1	21.6	20.0	—
b	—	1,173	3.3	34.2	10.4	48.2	9.2	30.0	21.5	20.0	6.4
Average of Tables 2 and 3											
a	—	1,136	3.5	26.7	13.7	70.9	—	29.8	19.1	17.5	—
b	—	1,122	3.4	27.4	13.7	45.7	—	28.7	19.0	17.5	6.1

[a] Estimated amount of corn grain fed in the Ohio M15 silage.

[b] Trials 1 to 4, inclusive, soybean oil meal; Trials 5 to 12, inclusive, grain mixture consisting of 3 parts corn and 1 part soybean oil meal.

show that the cows produced an average of 36.5 lb. of FCM on the Eureka silage ration and 37.2 lb. on the Ohio M15 ration. The amounts of TDN and dry matter were approximately the same on both silage rations, but the cows received the equivalent of 5.3 to 8.8 lb. (av. 6.6) of U. S. No. 2 corn per day when the Ohio M15 silage was fed.

In Trials 8, 9, and 10, Eureka silage was fed first, followed by Ohio M15, but in Trials 11 and 12, the silages were reversed. The summarized data show that the cows produced 32.8 lb. of FCM per day on the Eureka silage and 34.2 lb. on the Ohio M15. This difference was due primarily to the marked drop in FCM of the two cows used in Trials 11 and 12.

The over-all results obtained from the comparison of Eureka and Ohio M15 corn silages for milk production when fed either with or without a grain supplement are summarized at the bottom of Table 3. These results suggest a slight superiority of Ohio M15 silage for milk production (av. 27.4 vs. 26.7 lb. of FCM per day) on the same amount of TDN. Slightly more TDN was fed than required and 1.1 lb. less dry matter was consumed by the cows when the Ohio M15 silage was fed.

DISCUSSION

A comparison of Eureka (immature) and Ohio M15 (mature) corn silages in 10 trials without a grain supplement indicated a slight superiority of Ohio M15 silage for milk production per pound of dry matter intake. In 12 comparisons in which the silages were supplemented with soybean oil meal or a grain mixture, the amount of FCM produced per pound of dry matter intake was also slightly in favor of the Ohio M15 silage. The summarized data show that slightly less (1.1 lb.) dry matter was consumed by the cows when they received the Ohio M15 silage but that they consumed approximately the same amount of TDN. The average body weight of the cows tended to be slightly higher when they received the Eureka silage.

The close similarity of the milk-producing value of the dry matter content of the two silages is significant in view of the marked difference in the amount of U. S. No. 2 corn in the Ohio M15 silage. The cows consumed an average of 5.6 lb. per day compared to 0.76 lb. in the Eureka silage. In other words, the dry matter in the Eureka silage, an immature silage with very little grain, was about as valuable as the Ohio M15, a mature silage high in corn grain.

In a previous publication, Huffman *et al. (5)* have shown that some hays contain more milk-producing properties than can be explained on the basis of their TDN content when they replace all or part of the basal hay that was used to deplete the cows of their so-called unidentified factor(s). Using this depletion method, Huffman and Duncan *(6)* showed that when immature corn silage (Eureka) replaced part of the TDN in an all-hay ration, the amount of FCM increased. It appears, therefore, that the unidentified factor(s) needed to balance mature hays is present in the stalks and leaves of immature corn plants and that it resembles the milk-producing power of young pasture grasses. Barnett *(1)* has stated, as a result of the work of Percival *(14)*, that the fructosan content

of grasses may be a limiting factor in the production and digestibility of silage. Spring grasses that are high in protein and fructosans tend to make good quality silage, whereas young grasses cut later in the year, which are also high in protein but low in fructosans, tend to make poor silage. It is suggested that the digestibility of silage increases in direct proportion to the fructosan content of the original grass crop. A previous investigation *(3)* has shown that the corn grain present in corn silage is just as valuable for milk production as the same amount of corn grain fed as ground corn and cob meal.

When additional grain was fed as a supplement to the immature and mature silage rations, the amount of FCM was slightly in favor of the mature Ohio M15 silage in 7 of 12 trials. The cows used in Trials 11 and 12 were chiefly responsible for the difference in favor of the Ohio M15. The average body weights of the cows were slightly greater during the immature silage-feed periods. Hayden and Perkins *(4)* found that immature corn silage seemed to be more efficient for milk and fat production; however, the cows gained more in body weight when fed the more mature grain variety of corn silage. These results are essentially in agreement with those of Hayden and Perkins in that an immature corn silage with few ears and an early maturing variety with a large number of ears were about equal for milk production on a dry matter basis. They are also in agreement with the results reported by White and Johnson *(15)*, who compared early, medium, and late-maturing corn silages, on an equal dry matter basis, in rations containing an ample grain supplement and found no significant difference in milk production. The results reported in this paper also are in agreement with those of Nevens *(10)*, who observed slightly less milk production with immature corn silage, which may have been due to the cow's poor appetite for immature silages. The results also show that the dry matter content of immature silage has about the same grain-equivalent value for milk production as mature corn silage high in corn grain.

These results should not be construed to mean that the immature varieties of corn are recommended for silage. Nevens and Dungan *(11)* and Nevens *et al.* *(12, 13)* have pointed out that the early maturing, high-grain yielding varieties which mature before frost give the greatest return of nutrients per acre. The relative nutritive value of mature corn silage with few ears and mature corn silage high in ears is not known.

SUMMARY

Immature (Eureka) and mature (Ohio M15) corn crops grown in the same field were ensiled at the same time. Just prior to ensiling, the two varieties were randomized in order to calculate the yields per acre, dry matter content of the whole corn plant, yield of stalks, bushels of U. S. No. 2 corn, and pounds of corn per pound of silage.

The silages were compared in feeding trials without supplemental grain with milking cows. The amount of FCM produced was slightly in favor of the mature Ohio M15 silage, on the dry basis, but the difference was not considered significant. The estimated average amount of U. S. No. 2 corn consumed in the imma-

ture silage was 0.76 lb. per day, whereas the mature silage supplied 5.6 lb. per day. The results indicate that most of the grain-equivalent in immature corn silage was present in the vegetative part of the plant (stalks and leaves).

The immature and mature silages were also compared in 12 trials for milk production when the rations were supplemented with grain. These results also indicate that the mature corn silage was slightly superior to the immature silage, although the difference was not considered significant.

The importance of using varieties of corn for silage that mature before frost was pointed out.

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METABOLISM OF BULL SEMEN. III. RELATION OF LACTIC ACID AND ITS ACCUMULATION DURING INCUBATION WITH OTHER SEMEN QUALITY MEASUREMENTS AND NONRETURNS[1]

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In 1927, McCarthy *et al. (20)* observed the now well confirmed glycolytic ability of sperm with concomitant accumulation of lactic acid. Both manometric and chemical methods have been used. Glycolysis, as measured by lactic acid gain during storage or incubation, has been shown to be highly correlated with sperm concentration *(3, 4, 6)*, initial motility *(3, 4, 23)*, and storage motility *(4, 26)* but not appreciably with fertility *(3, 4)*. Glycolysis and respiration rates have shown a correlation of ± 0.77 for ram semen *(4)* on a sample within-ram basis.

Seminal sugar decrease recovered as lactic acid has been variable because of such factors as incubation time *(5, 6, 26)*, type of incubation *(5, 18, 19)*, type of diluent *(5, 19, 26, 28)*, extent of dilution *(5, 20, 26)*, and type of washing and suspension *(5, 21)*. These recoveries have generally been 25 to 83% under aerobic conditions *(5, 6, 19, 26)* and up to 91% under anaerobic conditions *(5, 19)*. In egg yolk citrate more lactic acid has accumulated than could be accounted for on the basis of seminal sugar *(28)*.

Sperm have been shown capable of utilizing lactic, pyruvic *(15, 19, 21)*, and acetic acid *(14, 19, 21)*. Lactic acid is present in freshly ejaculated bull semen *(2, 5, 6, 17)* and appears essentially unchanged during successive collections at short intervals *(17)*.

Since glycolysis proceeds preferentially to respiration in semen *(15, 16, 19, 26, 28)*, a comparison of lactic acid with other physical and metabolic characteristics of high quality semen was made for purposes of establishing variations and interrelationships.

METHODS

Semen samples collected between October, 1952, and January, 1953, from 35 bulls of four breeds in routine artificial service at Northwest Co-op Breeders, Burlington, Wash., were used. Handling of bulls and semen was as previously described *(8, 9, 11)*. Concentration of sperm was determined with a calibrated Leitz photelometer. Nonreturns were determined from first and second services (60- to 90-day), with 70% of the services occurring the day after collection.

Semen measurements before and after incubation for 1 hour at 37° C. were made for fructose, lactic acid, pyruvic acid, ascorbic acid, inorganic phosphorus,

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and motility. In addition, total phosphorus, and modified resazurin reduction time were determined. The following methods were used: *(a)* fructose—Roe *(24)* as modified *(8, 11)*; *(b)* pyruvic acid—Friedemann and Haugen *(12)*; *(c)* lactic acid—Barker and Summerson *(1)*; *(d)* ascorbic acid—Roe and Keuther *(25)*; *(e)* inorganic and total phosphorus—Fiske and SubbaRow *(10)*, as previously described *(11)*; and *(f)* resazurin reduction—Erb *et al. (7)*, modified by using Na citrate buffer instead of phosphate. Colorimetric determinations were made with an Evelyn colorimeter.

Immediately after collection of semen, 0.5 ml. was added to a 10 × 100 mm. test tube containing 2 ml. of 2.9% Na citrate buffer at 37° C. in a constant temperature water bath. One ml. of the diluted semen was removed immediately and mixed gently with 9 ml. of cold 10% trichloroacetic acid in order to determine zero time (initial) levels of the constituents studied. This process was repeated at 1 hour of incubation under aerobic conditions. Motility was determined before and after incubation on a 1 to 10 scale. Statistical analyses were according to Snedecor *(27)*.

RESULTS AND DISCUSSION

Measurements made on 261 semen samples from four breeds including averages by breeds, standard deviations, and standard error of means for between samples and samples within bulls are shown in Table 1; tests for significant variation between breeds and bulls within breed are also shown. Linear correlation coefficients, as determined by covariance methods, comparing initial lactic acid, lactic acid after incubation for 1 hour at 37° C., and lactic acid increase during incubation with other semen measurements are shown in Table 2 for bulls, breeds, bulls within breed, and samples within bull.

Important relationships between lactic acid and other semen measurements are shown in Figures 1 to 3.

Sperm concentration. Analysis of other portions of this study has shown a high sample within-bull relationship between concentration and total phosphorus ($r = 0.84$), a lesser but significant relationship with initial inorganic phosphorus ($r = 0.21$) *(11)*, and fructose utilization rates which were linear with sperm concentration ($r = 0.52$) *(8)* when sufficient fructose was available for maximum fructolysis. Sperm concentration is linearly correlated with lactic acid levels in semen after incubation for 1 hour (Figure 1) with a correlation of 0.62 for samples within bulls (Table 2). These observations are consistent with other studies *(3-6)*. Levels of lactic acid initially present in the semen (Table 1) agree with others *(2, 5, 6)* and show no important relationships with sperm concentration (Figure 1) or other semen characteristics (Table 2).

Pyruvic acid also increased (Figure 1) during incubation with increasing sperm concentration but in a less positive manner. Comparison of pyruvic acid after incubation for 1 hour and sperm concentration *(9)* showed a significant low order correlation ($r = 0.12$) and no relationship with increase in pyruvic acid during incubation.

TABLE 1

Breed averages, standard deviations (S.D.) and standard error of means (S.E.) for semen samples (total and within bull) and tests of significance for breeds and bulls within breed

Variable	Breed averages					Between samples		Sample within bull		Significance[a]	
	Guer.	Jer.	Hol.	Hereford	Total	S.D.	S.E.	S.D.	S.E.	Breed	Bulls within breed
Number of samples and degrees of freedom	91	57	92	21	261	261	261	226	226	3	31
Ascorbic acid—initial *(mg. %)*	8.93	8.63	6.66	9.23	8.09	2.82	0.17	2.14	0.14	NS	NS
—1 hour	8.33	8.00	5.79	9.09	7.42	2.93	0.18	2.17	0.14	**	**
—decrease	0.60	0.63	0.87	0.14	0.67	1.40	0.09	1.39	0.09	NS	NS
Pyruvic acid —initial *(mg. %)*	5.30	4.58	4.72	3.93	4.83	3.07	0.19	3.14	0.21	NS	NS
—1 hour	9.52	10.84	10.31	7.72	9.94	3.85	0.24	3.86	0.26	**	NS
—increase	4.22	6.26	5.59	3.79	5.11	5.93	0.37	5.81	0.39	*	NS
Lactic acid —initial *(mg/ml)*	0.51	0.53	0.46	0.40	0.49	0.16	0.01	0.14	0.01	**	**
—1 hour	2.29	2.87	2.71	2.06	2.54	0.66	0.04	0.55	0.04	**	**
—increase	1.78	2.34	2.25	1.66	2.05	0.62	0.04	0.52	0.03	**	**
Inorganic —initial *(mg. %)*	17.50	16.90	17.30	18.10	17.30	6.86	0.42	6.95	0.46	NS	NS
phosphorus—1 hour	18.30	18.70	20.10	20.30	19.20	6.72	0.42	6.79	0.45	NS	NS
—increase	0.80	1.80	2.80	2.20	1.90	6.13	0.38	6.16	0.41	NS	NS
Fructose —initial *(mg/ml)*	7.46	4.89	6.17	5.34	6.27	1.91	0.12	0.98	0.66	**	**
—1 hour	4.43	1.29	2.76	3.00	3.04	2.21	0.14	1.28	0.08	**	**
—decrease	3.03	3.60	3.41	2.34	3.23	0.90	0.06	0.75	0.05	**	**
Total phosphorus *(mg/ml)*	1.25	1.62	1.50	1.26	1.42	0.42	0.03	0.34	0.02	**	**
Resazurin reduction time *(min.)*	6.68	3.61	4.88	6.81	5.39	5.40	0.33	5.32	0.35	**	NS
Sperm concentration *(10^9/ml)*	1.39	1.98	1.73	1.56	1.65	0.56	0.03	0.38	0.03	**	**
Motility —initial *(rating)*	7.26	8.42	7.80	7.10	7.69	1.34	0.08	1.06	0.07	**	**
—1 hour	6.55	7.28	7.51	5.48	6.96	1.93	0.12	1.56	0.10	**	**
—decrease	0.71	1.14	0.29	1.62	0.73	1.79	0.11	1.64	0.11	**	**
Nonreturns *(%)*	70.70	73.80	72.60	70.30	72.00	9.15	0.57	8.46	0.56	NS	**
Fructose decrease recovered as lactic acid *(%)*	58.70	65.00	66.00	70.90	63.50	16.94	1.05	16.63	1.10	*	NS

[a] Analysis of variance for $n = 261$; NS = not significant; * = $P < 0.05$; ** = $P < 0.01$.

TABLE 2

Analyses of covariance

Measurement	Linear correlation coefficients														
	Total	Bulls	Breeds	Bulls within breed	Residual	Total	Bulls	Breeds	Bulls within breed	Residual	Total	Bulls	Breeds	Bulls within breed	Residual
	Initial lactic acid					Lactic acid after incubation					Lactic acid increase				
Degrees of freedom	260	34	3	31	226	260	34	3	31	226	260	34	3	31	226
Lactic acid —initial	—	—	—	—	—	0.31[a]	0.35[b]	0.33	0.38[b]	0.29[a]	0.09	0.11	0.20	0.16	0.03
—1 hour	0.31[a]	0.35[b]	0.33	0.38[b]	0.29[a]	—	—	—	—	—	0.97[a]	0.98[a]	0.99[a]	0.97[a]	0.97[a]
—increase	0.09	0.11	0.20	0.16	0.03	0.97[a]	0.98[a]	0.99[a]	0.97[a]	0.97[a]	—	—	—	—	—
Ascorbic acid—initial	−0.04	−0.01	0.40	−0.14	−0.06	−0.12[b]	−0.22	−0.57	−0.04	−0.04	−0.12[b]	−0.23	−0.65	0.00	−0.02
—1 hour	−0.06	0.01	0.33	−0.11	−0.11	−0.20[a]	−0.29	−0.60	−0.10	−0.13[b]	−0.20[a]	−0.31	−0.67	−0.08	−0.11
—decrease	−0.05	0.05	−0.13	0.09	−0.08	−0.18[a]	−0.31	−0.67	−0.22	−0.15[b]	0.18[a]	0.34[b]	0.67	0.26	0.13[b]
Pyruvic acid —initial	−0.12[b]	−0.07	0.52	−0.21	−0.14[b]	−0.11	−0.15	−0.28	−0.11	−0.11	−0.09	−0.15	−0.37	−0.15	−0.08
—1 hour	−0.07	0.28	0.59	0.16	−0.15[b]	0.13[b]	0.59[a]	0.93	0.37[b]	0.00	0.16[a]	0.57[a]	0.88	0.36[b]	0.04
—increase	0.10	0.56[a]	0.32	0.88[a]	−0.04	0.22[a]	0.89[a]	0.99[a]	0.81[a]	0.03	0.22[a]	0.85[a]	0.99[a]	0.72[a]	0.10
Fructose —initial	−0.10	−0.13	0.04	−0.19	−0.10	−0.36[a]	−0.45[a]	−0.64	−0.33[b]	−0.30[a]	−0.35[a]	−0.45[a]	−0.65	−0.30	−0.29[a]
—1 hour	−0.15[b]	−0.20	−0.13	−0.23	−0.14[b]	−0.62[a]	−0.68[a]	−0.82	−0.57[a]	−0.63[a]	−0.62[a]	−0.67[a]	−0.84	−0.56[a]	−0.62[a]
—decrease	0.15[b]	0.27	0.53	0.17	0.10	0.76[a]	0.90[a]	0.95[b]	0.87[a]	0.68[a]	0.76[a]	0.90[a]	0.91	0.89[a]	0.68[a]
Inorganic P —initial	0.14[b]	0.06	−0.78	0.12	0.16[b]	0.12[b]	0.15	−0.86	0.31	0.12	0.09	0.15	−0.78	0.34[b]	0.08
—1 hour	0.11	0.09	−0.85	0.27	0.12	0.19[a]	0.32	0.22	0.38[b]	0.17[a]	0.17[a]	0.32	0.35	0.34[b]	0.14[b]
—increase	−0.03	0.03	−0.58	0.15	−0.05	0.08	0.18	0.58	0.06	0.05	0.09	0.18	0.68	0.02	0.07
Motility —initial	0.24[a]	0.37[b]	0.44	0.35[b]	0.18[a]	0.62[a]	0.75[a]	0.95[b]	0.67[a]	0.52[a]	0.59[a]	0.72[a]	0.92	0.63[a]	0.49[a]
—1 hour	0.02	−0.03	0.25	−0.11	0.04	0.47[a]	0.62[a]	0.91	0.50[a]	0.37[a]	0.49[a]	0.66[a]	0.91	0.57[a]	0.38[a]
—decrease	−0.16[a]	−0.41[b]	−0.12	−0.48[a]	−0.08	0.05	0.11	0.27	0.04	0.02	−0.09	0.20	0.30	0.16	−0.23[a]
Resazurin time	−0.12[b]	−0.16	−0.31	−0.10	−0.11	−0.71[a]	−0.82[a]	−0.97[b]	−0.74[a]	−0.64[a]	−0.37[a]	−0.83[a]	−0.96[b]	−0.77[a]	−0.23[a]
Total phosphorus	0.10	0.16	0.22	0.14	0.06	0.68[a]	0.78[a]	0.97[b]	0.68[a]	0.61[a]	0.69[a]	0.81[a]	0.98[b]	0.73[a]	0.60[a]
Sperm concentration	0.10	0.10	0.18	0.08	0.11	0.66[a]	0.73[a]	0.89	0.66[a]	0.62[a]	0.67[a]	0.75[a]	0.90	0.69[a]	0.61[a]
Nonreturns	0.08	0.04	0.33	−0.01	0.09	0.14[b]	0.43[a]	0.98[b]	0.35[b]	0.09	0.13[b]	0.79[a]	0.97[b]	0.37[b]	−0.05

[a] Significant at 1% level of probability (degrees of freedom − 2).

[b] Significant at 5% level of probability (degrees of freedom − 2).

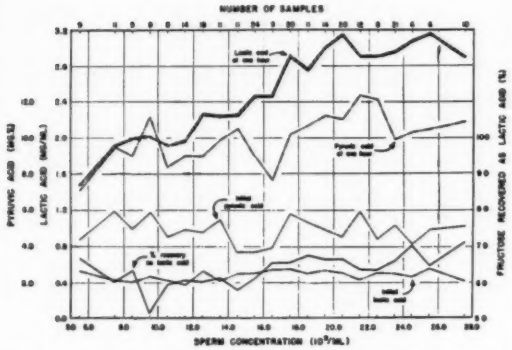

FIG. 1. Relation of increasing sperm concentration with pyruvic and lactic acid content and fructose loss recovered as lactic acid.

Fructose decrease during incubation accounted for as lactic acid was consistently higher, on the average, for semen samples exceeding 1.6×10^9 sperm per milliliter. This does not appear to be a breed difference, since the two breeds (Hereford and Guernsey) showing the lowest average sperm concentration had the highest and lowest recovery rates (Table 1). The more highly concentrated samples rated higher in initial motility *(9)* for samples within bull ($r = 0.44$ for 365 d.f.). Relation of sperm concentration and fructose recovery as lactic acid

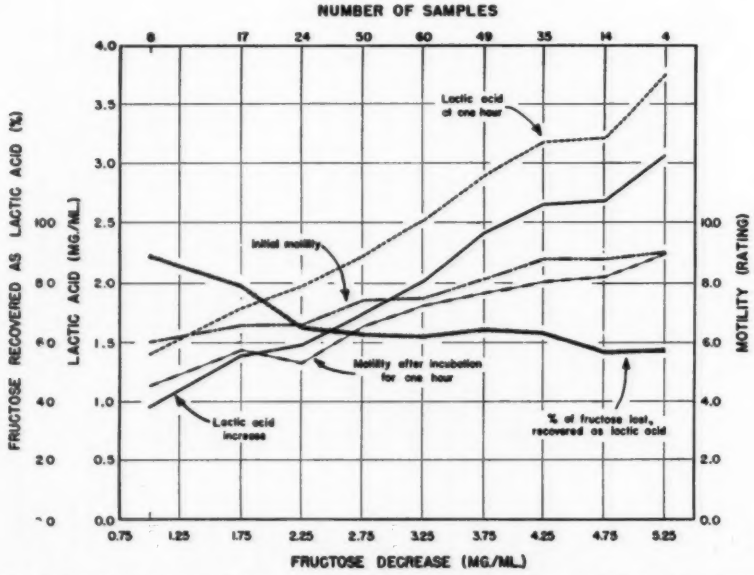

FIG. 2. Relation of fructose decrease after incubation for one hour at 37° C. with changes in sperm motility and lactic acid and recovery of fructose as lactic acid.

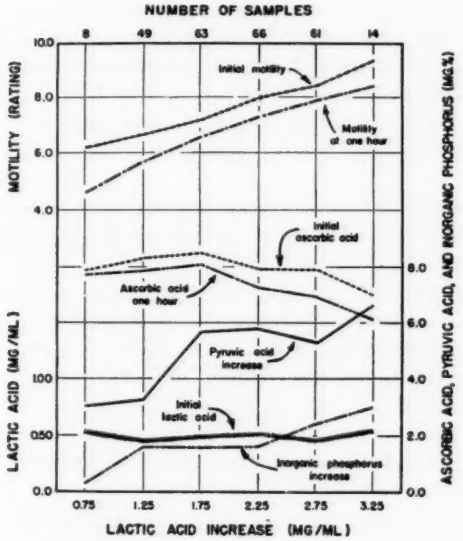

FIG. 3. Relation of lactic acid increase after incubation for one hour at 37° C. with ascorbic acid and motility decrease, pyruvic acid and inorganic phosphorus increase.

is biased by sperm activity, since recovery increased from 61 to 66% as initial motility ratings increased from 6 to 10.

Fructose decrease during incubation. The relationship between initial motility and lactic acid accumulation is clearly shown in Figures 2 and 3. Semen samples accumulating the most lactic acid showed the highest average initial motility and the least average decline during incubation. This is in contrast with an average motility rating decline of 1.5 during incubation for 79 samples rating 9 and 10 initially and only 0.4 of a rating for 129 samples initially rating 6 and 7.

The within-bull correlation for lactic acid increase and decline in motility during incubation (Table 2) was –0.23 ($P < 0.01$), which further describes the relationship shown in Figures 2 and 3. These results are interpreted to indicate that lactic acid accumulation does not inhibit motility at levels reached under the conditions of this study. This appears to be in agreement with Salisbury *(26)*, who observed storage motility stimulation from added glucose, and the Lardy and Phillips *(13)* finding that energy for motility is obtained through glycolysis.

Though the correlation between fructose loss and lactic acid increase is high (Table 2), the recovery of fructose as lactic acid declines from over 80% for 25 samples using less than 2.0 mg. of fructose per milliliter during 1 hour of incubation to below 60% for 18 semen samples utilizing more than 4.5 mg. of fructose. These samples probably represent metabolic extremes. The low fructose utilizing group was below average in sperm concentration and below average in initial

and 1-hour levels of pyruvic acid, suggesting faulty glycolysis. Motility decline during incubation was more than double the rate expected for these samples, which averaged 6.4 in initial motility. Ample fructose (over 6.50 mg. per milliliter) remained after incubation. The reverse was true for samples with high fructose utilization where fructose frequently was inadequate to support maximum glycolysis *(8)*. These latter samples also showed less accumulation of pyruvic acid.

Lactic acid increase. Relatively small differences exist between ascorbic acid and lactic acid (Table 2 and Figure 3) though ascorbic acid loss was significantly correlated with lactic acid increase during incubation ($r = 0.13$). It should be recalled that the method *(25)* used for this study measures total ascorbic acid and does not distinguish between the reduced and oxidized forms. The ranges observed for ascorbic acid are higher but not inconsistent with a report by Phillips *et al. (22)*, who used a method determining only reduced ascorbic acid. Pyruvic acid increased markedly (Figure 3) for the samples accumulating in excess of 1.5 mg. of lactic acid compared with those accumulating lesser amounts (57 samples). This relationship is not linear; hence the correlations shown in Table 2 are probably too low.

Nonreturns. Correlations between the lactic acid measures and nonreturns (Table 2) are of a low order and not significant for samples within bulls. However, significant correlations for bulls, breeds, and bulls within breeds were observed for lactic acid levels after 1 hour of incubation and for lactic acid increase during incubation. The between-bull within-breed correlations are similar to those observed for fructose utilization *(8)*. Initial lactic acid levels showed no relation with nonreturns. These findings confirm the results from previous studies *(3, 4)*. Fructose recovery as lactic acid was not related to nonreturns for bulls, breeds, bulls within breeds, or samples within bulls.

SUMMARY

Lactic acid was determined initially and after incubation for 1 hour at 37° C. under aerobic conditions on 261 semen samples from 35 bulls of four breeds whose 60- to 90-day nonreturns averaged 72%.

High positive correlations on a within-bull basis were observed for lactic acid level at 1 hour and increase when compared with fructose loss, sperm concentration, total phosphorus, initial motility, and motility after incubation.

High negative correlations were observed for fructose levels after incubation, resazurin reduction time, and motility decline during incubation. Initial lactic acid was not highly correlated with other measures employed.

Highly significant between-bull correlations between nonreturns and lactic acid levels after incubation ($r = 0.43$) and lactic acid increase ($r = 0.79$) were observed. No relationship existed for samples within bulls.

Fructose loss during incubation recovered as lactic acid averaged 63.5%. High lactic acid accumulation was associated with above-average maintenance of motility during incubation.

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CHANGES IN TYPE CLASSIFICATIONS OF DAIRY CATTLE[1]

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The sources of error in a subjective measurement such as type classification are comparable in kind to those to which an objective measurement is liable. However, the magnitudes of the errors from the various sources may be different. Unconscious changes in the mental ideal or scale used by the classifier correspond to imperfections in the instruments used for objective measurements. Carelessness by the classifier or his recorder is similar to incorrect placement or reading of the measuring instrument or to incorrect recording. Changes that occur in the animal's appearance, especially if the interval between classifications is long, correspond to differences in the position of an animal when it is measured at various times and are errors only in the statistical sense where the "true" measurement is that which would be approached by the average of an indefinitely large number of measurements repeated under similar circumstances.

Type classifications are only moderately repeatable. Johnson and Lush *(5)* found a correlation of 0.34 when Holstein females were rated at yearly intervals by different classifiers. This correlation included ratings of all females over 10 months of age. Hyatt and Tyler *(3)* found 0.73, 0.82, and 0.62 for three different classifiers when the same man classified Ayrshire cows at intervals of 4 months and 0.55 when different men classified the cows. In the latter case, the intervals between classifications were longer. Hyatt and Tyler *(4)* also found the correlation between the average of several ratings of each Ayrshire heifer before first calving and the average of the first and second ratings after calving to be 0.44. These studies gave little indication of why repeatabilities were no larger, although such things as age of the cow and interval between ratings were examined.

Benson *et al. (1)* used two official classifiers twice yearly to classify Ayrshire cows and heifers. One classifier returned for each classification, and the other was changed each time. Correlations for cows were 0.56 for the same classifier at different times, 0.61 to 0.76 for different classifiers at the same time, and 0.48 for different classifiers at different times. For heifers the corresponding correlations were 0.28, 0.54 to 0.81, and 0.20.

Touchberry and Tabler *(7)* analyzed some of the causes of imperfect repeatability by having the same three men classify Holsteins and Guernseys simultaneously but independently at two dates about 10 months apart. The intrabreed correlation between ratings of cows made by the same man on different dates was 0.40; that for different men on the same date was 0.62; and for different men on different dates the correlation was 0.37. Changes in the appearance of

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the cow from one time to the next were a major cause of changes in ratings. Disagreements between the ideals of the classifiers were small.

Harvey *et al. (2)* in a similar analysis of Jersey and Holstein heifers classified at 6-month intervals to about 24 months of age found a correlation of 0.40 when the same classifier rated the heifers at different times, 0.64 when different classifiers rated the heifers at the same time, and 0.37 for different classifiers working at different times. The agreement between the results of Touchberry working with cows and those of Harvey working with heifers and a different set of classifiers is remarkable.

The present study is similar in design to those of Touchberry and Harvey. The major differences are that the classifiers for this study were trained in different schools and have met infrequently so that they have had little opportunity to observe each other's scoring, and both cows and heifers were classified at yearly intervals for 3 years.

DATA STUDIED

All animals that were in the Iowa State College Holstein-Friesian herd on each classification day and were more than about 4 months old were included in classifications in May or June of 1953, 1954, and 1955. The herd has been closed to outside animals for about 20 years. The average inbreeding coefficient, relative to about 1910, was about 12%. The reduction in variability expected from this should scarcely be noticeable except for highly hereditary characteristics. All heifers were kept until they were at least nearly through their first lactation. Culling was done according to a selection index based about three-fourths on production and one-fourth on type. Actually this meant only mild selection for type, since type turned the decision on whether to cull a cow only when she was near the culling level on her production records.

The classifiers were men of long experience in judging cattle and had separately attended and participated in numerous classification demonstrations. At one time or another, they had been official classifiers for one or more of the breed associations.

The classifiers were asked to classify the animal just as they would under the official system with two exceptions. First, they designated whether the cow was in the high, low, or middle part of the standard classes in which they had placed her. Second, they classified the heifers that had not yet calved the first time. This was extended down to ages as young as they would venture any opinion, which was usually about 4 months. The classifying was done without the classifiers consulting each other. Cows were led one by one before each of the three men stationed out of sight and sound of each other. When the animals that were on pasture were rated, the classifiers were within sight of each other but were out of earshot. A clerk recorded for each classifier, and the classifier wrote nothing.

The 18 possible type ratings were coded consecutively from 0 for "Low Poor" to 17 for "High Excellent." The computational methods were mainly the analy-

sis of variance, but some aspects were studied also by correlations. Because of the possible importance of differences in age, the data were divided into groups according to whether the classification happened before or after the animal had calved for the first time. Group 1 included the 65 cows that had calved before any of their ratings were made. Group 2 included 57 heifers that were rated at least once before first calving and at least once afterward. Group 3 included 92 heifers that had not yet calved even when their latest rating was made. For the correlation study, Group 4 included the 30 cows that had calved before their first classification and were classified three times. All of these were in Group 1, but Group 1 also included cows that were in the herd only one or two years. Group 5 included the 44 cows that were all classified three times with at least one of those times being before they had calved and at least one being after first calving. These were therefore included in Group 2, but Group 2 also included some animals that were classified in the first two years but had left the herd before the 1955 classification.

RESULTS

In Table 1 are shown the amount of evidence and the size of each mean square in the analysis of variance. Disproportionality of the numbers in the classes makes the tests of significance only approximate. The approximation is close except, perhaps, for the differences between judges and between years. The error term used for testing those was the highly significant JY mean square, based on only 4 degrees of freedom.

The components of variance derived from Table 1 are shown in Table 2. These show comparably the average amount of variance coming from each different source, whereas the mean squares in Table 1 cannot be so compared because the components are in them various numbers of times.

The means for Groups 1, 2, and 3 were 6.46, 6.18, and 6.56, respectively, and the standard deviations were 2.33, 1.77, and 1.63. These figures are lower than for the 23,024 Holsteins classified officially in 1954, which averaged 8.55 and had

TABLE 1

Analysis of variance in final type classifications

Source of variance	Group 1. Cows rated after calving		Group 2. Heifers rated before and after calving		Group 3. Heifers rated before calving	
	(d/f)	*(M. sq.)*	*(d/f)*	*(M. sq.)*	*(d/f)*	*(M. sq.)*
Cows	64	22.74*[a]	56	9.49**	91	4.71*
Judges	2	5.80	2	1.32	2	21.08
Years	2	41.27	2	65.04**	2	29.20
C × J	128	1.48	112	1.21	182	1.55
C × Y	71	2.99**	94	3.72**	48	2.63**
J × Y	4	12.18**	4	8.35**	4	5.58**
Residual	142	1.22	98	0.90	96	1.30

[a] * indicates statistical significance at the level $0.01 < P < 0.05$.
** indicates statistical significance at the level $P < 0.01$.

These are approximate, as the error term used for testing a primary effect was the larger of the two interactions involving that effect, e.g., in Group 1 the 22.74 for cows was tested against the 2.99 for *CY*, but the 41.27 for years was tested against the 12.18 for *JY*.

TABLE 2

The components of variance in the mean squares in Table 1

Component for		Group 1 After calving		Group 2 Before and after first calving		Group 3 Before first calving	
		Actual units	Per cent of total	Actual units	Per cent of total	Actual units	Per cent of total
Cows	(C)	3.02	55	0.66	21	0.27	10
Judges	(J)	−0.06	—	−0.05	—	0.10	4
Years	(Y)	0.19	3	0.35	11	0.16	6
C × J	(CJ)	0.07	1	0.11	3	0.08	3
C × Y	(CY)	0.65	12	0.96	31	0.58	22
J × Y	(JY)	0.25	5	0.15	5	0.09	4
E	(E)	1.27	23	0.91	29	1.38	52
Total[a]		5.45		3.14		2.66	

[a] The negatives were insignificant and were considered zero.

a standard deviation of 2.44, after making Sheppard's correction for coarseness of grouping. Also, Rennie and Raithby *(6)* found a mean of 8.35 for nearly 13,000 Holsteins classified officially in Canada, with 2.05 for the standard deviation after Sheppard's correction. As compared with those classified officially, the I.S.C. herd thus was lower in type and somewhat more uniform. However, the cows classified officially were somewhat selected, since classifying a herd is optional with the owner, and, also, some intraherd culling may have been done before classification day. Whether such selection was enough to make the official average higher and the standard deviation lower than for an entirely representative sample of the breed by a considerable amount or by only an extremely small amount seems impossible to determine.

That type classifications varied more among older and less among younger animals is shown in the bottom line of Table 2. Almost all of this difference is in the cow component (line 1), the totals of the other six components differing by only 0.09 or less. Presumably the differences between young animals have not yet developed fully, or else the judges merely felt less certain about the young animals and, therefore, stayed closer to the herd average when rating them. The cow component measures the variance caused by real differences between individual animals, the definition of "real" being that all three judges agreed on the differences and they were the same each time the animals were rated. The cow component was more than half of the variance in Group 1, a fifth of that in Group 2 and a tenth in Group 3.

The component between judges measures the extent to which the ratings given by each judge over all 3 years were at an average level differing from the corresponding average levels of the other judges. This was practically zero, insignificantly negative in two groups and insignificantly positive in Group 3. This means merely that the three judges had almost identical general levels for their standards. In all kinds of subjective measurements—not merely in type classification—the observer, being aware of the usual range of the ratings, may unconsciously be under some compulsion to offset high ratings with low ones and thus to make the general average of all his ratings come close to some fixed level.

By itself such an element in the rating process would tend to produce negative intraclass correlations and negative components between judges. Consequently, the reasons that the differences between the general levels of the judges are so very small may be complex and cannot be identified here with any assurance. For practical purposes it is enough to know that they are tiny. That was also true in the Illinois and Idaho data, where general differences between judges contributed nothing to the variation.

The component *(Y)* for years measures the extent to which the general average of the ratings differed more from year to year than would be expected from the effects of *E*, *CY*, and *JY* on them. This component was moderately large in Group 2. The possible causes of such differences are varied. The average appearance of the animals may have changed from one year to another because of prior environmental conditions, such as condition of pastures and annoyance by flies. The judges might all have been influenced, without being aware of it, into a more pessimistic or more optimistic frame of mind by prevailing conditions of temperature, raininess, etc., on one day than on others. The composition of the groups changed a little, since some cows that were in Group 1 the first or second year were not in it the third year, some in Group 2 were classified in only 2 years, and some in Group 3 were first classified in the second or third year. However, the composition of the groups changed least in Group 2, which shows the largest difference between years.

The cow-judge interaction *(CJ)* measures the extent to which one man always rated a given cow consistently higher or lower than the other men did. It can be considered to measure the genuine disagreement in ideals among the judges. This sort of thing is often discussed among those interested in the show ring when they comment on one judge preferring one type and other judges preferring other types. This interaction did not even begin to approach statistical significance in the present data. It varied only from 0.07 to 0.11, which is no more than 3% of the total variance, even among the young animals. It was also small in the other studies, being only 11% and 3% of the totals at Illinois and 4% and 0% at Idaho.

The cow-year interaction measures the extent to which all three judges agreed that a cow should rate higher or lower in one year than in another. It is a measure of real changes in the appearance of the cow, the test of "real" being that all three judges agreed on the change. Among the causes of imperfect repeatability, i.e., after excluding the cow component, *CY* was the largest in Group 2 and second in Groups 1 and 3. Part of its large size in Group 2 may well be the changes in appearance of the udder, which are not evident before first calving. In this group every animal had at least one rating before calving and at least one afterward. However, other characteristics are also changing rapidly at this age. Hence, one cannot be positive that changes in appearance of the udder were actually responsible for much of the large cow-year interaction in this group. The cow-year component was also large in the Illinois experiment, 42% and 21% of the totals for the Holsteins and Guernseys, respectively. It was large in the West Virginia studies, as evidenced by the larger correlation be-

tween contemporary ratings by different men than between ratings by the same man at different dates. At Idaho this interaction was responsible for 27% and 16% of the variance. Clearly, the changes in a heifer's or cow's appearance from time to time are large and differ from cow to cow. Imperfectly discounted effects of being in different stages of lactation could have contributed to the C component when the calving interval was close to 12 months but to CY when the calving interval was much longer.

The judge-year component (JY) measures the extent to which the ratings by one man in one year were genuinely higher or lower than his average ratings in other years, after making due allowance for how much the average ratings of all three men went up or down together in the same years. This interaction is small in amount—about 5% each year. Interpretation of this component has some of the same uncertainties as the general component between judges. It was negative in the Illinois data. At Idaho it was 0% and 2% of the variation. Not much error comes from this source into type classification data. It might at times be important in differences between herd averages (where the averaging would cancel many of these other sources down to unimportance), but not in differences between individual cows.

The residual term (E) or triple interaction or "error" is a little more than half of all causes of imperfect repeatability in Groups 1 and 3 and is 37% in Group 2. It was 15% and 17% of the totals in the Illinois data and 28% for each breed at Idaho. The residual variation may well have many diverse causes but cannot be sub-divided further. Considerations like those on which "Sheppard's correction" is based suggest that only about 1/12 to 1/16 of E comes from coarseness of grouping (i.e., from rating a cow only to the nearest third of a type class). The judge's standards may fluctuate at least a little. Changes in the appearance of an animal from one year to another may appear worse to some judges than to others. Difficulties intrinsic to any process of integrating opinions about many parts of an animal to reach always the same over-all rating of net merit may be a large part. This residual term yields a standard error of about one-third of an official grade. That the error is this small suggests strongly that the official system does not utilize fully the sharpness of discrimination which its classifiers could express, provided they agree as well as these men did with each other and with themselves at different times.

CORRELATION ANALYSIS

A correlation between the ratings of a group of cows by one man at one time and the ratings of the same cows by the same man at another time or by another man at the same or other times by-passes the J, Y, and JY components of variance, since the things being correlated are deviations of an animal's rating from the average rating of that group at that time by that man. Also, a product-moment correlation measures the degree of likeness more accurately than does a correlation based on components of variance if the variance is heterogeneous within the various subgroups. All averaging of correlations was by the z transformation.

TABLE 3

Correlations between ratings of the same cow

		1953			1954			1955		
		J_1	J_2	J_3	J_1	J_2	J_3	J_1	J_2	J_3
1953	J_1	—	0.88	0.82	0.44	0.81	0.67	0.70	0.66	0.71
	J_2	0.38	—	0.82	0.46	0.48	0.60	0.56	0.56	0.69
	J_3	0.50	0.44	—	0.46	0.61	0.60	0.58	0.63	0.60
1954	J_1	0.33	0.26	0.31	—	0.51	0.65	0.31	0.33	0.54
	J_2	0.06	0.12	0.35	0.47	—	0.81	0.47	0.61	0.59
	J_3	0.08	0.12	0.45	0.49	0.72	—	0.51	0.71	0.72
1955	J_1	0.26	0.28	0.34	0.64	0.38	0.49	—	0.71	0.62
	J_2	0.11	0.22	0.30	0.46	0.50	0.55	0.64	—	0.62
	J_3	0.18	0.22	0.42	0.61	0.42	0.59	0.73	0.74	—

The figures above and to the right of the empty diagonal pertain to Group 4, consisting of 30 cows each classified three times after calving. The figures below and to the left of the empty diagonal pertain to Group 5, consisting of 44 cows each classified three times, at least one of which was before and at least one of which was after first calving.

In Table 3 are shown the correlations between the ratings of the same cow by the same judge in different years or by different judges in the same or in different years. On such small numbers of individuals the sampling errors of the correlations are large, of course, the standard error of z being about 0.19 in Group 4 and about 0.16 in Group 5. Hence, the differences between these individual correlations have too large sampling errors to be interpreted with assurance. However, not one of the 72 correlations is negative. If the variations among them are only sampling variations, clearly the value around which they are varying is positive and rather large.

The average of all 36 correlations in Group 4 is 0.63 and in Group 5 is 0.41. Almost surely, this difference is highly significant statistically, although there is some uncertainty about its precise sampling error. Agreement is closer on

TABLE 4

Summary of some average correlations

	Same judge, different times	Different judges	
		Same time	Different times
Group 4, product-moment method	0.57	0.74	0.60
Group 5, product-moment method	0.41	0.58	0.32
Group 1, by variance components	0.62	0.73	0.60
Group 2, by variance components	0.29	0.61	0.25
Group 3, by variance components	0.15	0.37	0.11
W. Virginia (1948)	0.62-0.82		0.55
W. Virginia (1951) mature cows	0.56	0.61-0.76	0.48
W. Virginia (1951) heifers	0.28	0.54-0.81	0.20
Illinois			
Holstein cows	0.41	0.73	0.30
Guernsey cows	0.54	0.76	0.51
Idaho			
Holstein heifers	0.31	0.59	0.29
Jersey heifers	0.44	0.67	0.40

ratings of older cows than on ratings of younger cows classified both before and after first calving. Table 4 shows in separate columns the average correlations between the ratings of the same cow by: *(a)* the same man, necessarily at different dates; *(b)* different men on the same day; and *(c)* different men on different dates. In *(a)* the sources of imperfect repeatability are only those described as *CY* and *E* in the analysis of variance. In *(b)* the only sources are *CJ* and *E*. In *(c)* they are *CJ*, *CY*, and *E*. In every line of Table 4 the figures for *(b)* are much the highest and (except in line 1) the figures for *(a)* are higher, but only slightly so, than the figures for *(c)*. Clearly, the judges agreed with each other more closely on the same date than each agreed with himself at different times. The agreement of a man with himself at different times is only a tiny bit higher than his agreement with other men at different times. This confirms, in terms of correlation, the finding from the analysis of variance that the *CY* component is large and the *CJ* component is small. Real changes in the animal's appearance from time to time are an important cause of imperfect repeatability of type ratings. Genuine disagreements of ideals among judges are an unimportantly small source of imperfect repeatability, at least among experienced judges such as those participating in these experiments.

The close agreement of line 1 with line 3 and of line 2 with line 4 is not surprising, since many of the animals are the same. Opportunities for disagreement in those cases arise only because Group 1 included also some cows which were rated only once or twice and Group 2 included some which were rated only twice —once before and once after calving. Also, any effects (presumably slight) of the variance not being homogeneous in different subgroups would have made the figures in lines 3 and 4 a bit lower than those in lines 1 and 2.

In the rest of Table 4 is shown the West Virginia, Illinois, and Idaho evidence on different animals and different judges, but bearing on the same points. The good agreement, considering the amounts of evidence, indicates strongly the general truth of the conclusion that cows change importantly in type from time to time but that disagreements in ideals between experienced judges are very small. The contrast between cows and heifers at West Virginia also parallels rather closely that between Group 1 and Group 3 or 2. This indicates general applicability of the not surprising conclusion that differences in type between young animals are smaller than those between mature animals, or at least the judges are less certain of them.

Averages of the correlations in Table 3 do not show that any one of the three judges was clearly more consistent with himself from one time to another than were the others. Judge 3 did have the highest average repeatability, 0.64 for Group 4 and 0.49 for Group 5. However, Judge 2, who was second with 0.55 on Group 4, was third with 0.29 on Group 5, and Judge 1, who was third with 0.50 on Group 4, was second with 0.43 on Group 5. Neither did the judges agree with each other more closely on consecutive years than on years far apart. In Group 4 the average agreement of a man with himself was 0.54 for consecutive years but was 0.62 between years one and three. In Group 5 the difference went in the other direction, their agreement averaging 0.45 on consecutive years but

0.30 for ratings 2 years apart. Agreement between different men averaged 0.58 on Group 4 for consecutive years and 0.64 for ratings that were 2 years apart. On Group 5 the corresponding figures were 0.22 and 0.49. In Group 4 the agreement between contemporaries was highest in the first year and lowest in the third year, but this was reversed in Group 5.

DISCUSSION

Although disagreements between classifiers both in general level *(J)* and in ideals of type *(JC)* were small in this experiment, such disagreement might be even less among official classifiers of any breed, if the frequent training sessions actually do standardize the work of those classifiers more than the ideals of these three judges had been standardized by their previous experiences.

Perhaps the agreement between judges on the same day was increased by presenting the animals to each judge in the same order, although it is not certain that any unconscious trends during the day in each judge's standard would be in the same direction, nor that all judges would react alike to a sudden change, such as a poor cow coming immediately after a very good one or the reverse. The order of presentation of individual cows in different years was not the same, but each year the cows in milk were classified before the dry cows and the heifers. The effect of order of presentation has not been measured, but some people think it is important. This would be difficult to measure except through photographs.

Heifers are evaluated less accurately than cows. *C* is smaller among the younger animals, but the sum of the other six components is nearly the same. The incomplete development of the udder at young ages may be a major factor in making the differences smaller at younger ages. However, judges are not accustomed to classifying heifers and they have no uniform standard for this, such as exists for cows. The increase in agreement between classifiers as the animals become older is illustrated by the average correlations of the classifiers with each other at the same time in Group 5. In 1953, Group 5 consisted entirely of uncalved heifers, and this correlation averaged 0.44. In 1954, some had calved for the first time, and the correlation averaged 0.57. In 1955, all had calved at least once, and the average correlation was 0.71.

A breeder interested in improving the type of his herd could increase his progress by rating his heifers and by basing a part of his selection on them. But he would not want to do much of this because: *(a)* Except when buying to assemble a herd, he is limited in his selection among heifers if he is to keep much freedom to select among the cows after their type is more clearly unfolded and they are beginning to show their differences in productiveness; *(b)* the repeatability of type differences is low at the young ages, and he would make small improvement in type, even if he selected each on an average of its classification at several times spaced several weeks or months apart.

The small size of the *C* component in young animals suggests that precalving classification should remain outside the official breed program. It would achieve some genuine discrimination, but the amount of that would be small and the

confusion between it and the more reliable post-calving ratings might outweigh any good that might be accomplished by making the pre-calving ratings official.

It is imaginable that a part of the CY component may really be genetic. That is, some of the genes in which they differ may affect appearance at different ages. Some breeders might want their animals to appear best as calves, others as heifers about to calve, others as mature cows, etc., according to the age at which they sell most of their surplus, or for advertising or other reasons. To the extent that CY really is genetic, a breeder would want his animals classified at the age when he wants them to appear at their best, so as to make most accurate the selection for whatever genes improve appearance most at that age. However, CY may result mostly or even wholly from temporary variations in previous environment, health, etc., which have happened to some animals and not to their contemporaries. That part of CY is really error, in every sense of the word, but could not be diminished by having several men classify the animal at the same time and using the average of their ratings. The design of this experiment did not permit separating CY into genetic and environmental portions.

How much accuracy is gained by classifying the cows several times and selecting each cow on the average of all n of her ratings? If the same man does the classifying, but at different dates, the E, Y, CY, and JY components in the average are reduced to $1/n$ of their values. If those values are as in Group 1 in Table 2, this would make selection on an average of two ratings about 1.13 and on an average of three ratings about 1.19 times as effective as selection on one rating. If the n classifications are done at a single date by n different men the E, J, CJ, and JY components would be reduced and the gain is less, the corresponding figures being 1.10 and 1.14. Because the J and CJ components are so small, the gain from having the classification done by a different man each time is very little more than from having it done by the same man at different times. In any case the major gain is from the reduction in E. A noticeable amount comes also from CY, JY, and Y if the classifications are on different dates.

In the present experiment the classifications were a year apart. The components involving Y might be smaller if the time intervals between classifications were shorter, or they might be more if some were in the summer and others in the winter. However, the close agreement of these results with those at Illinois and Idaho suggests that shortening the interval would make little difference—at least down to intervals of 4 or 6 months.

The gain from selecting on an average of n ratings is rather small (although real) where repeatability is as high as it was in Group 1. Such gains would be relatively larger within populations such as Groups 2 and 3 but, as mentioned earlier, it would rarely if ever be practical to cull more than a small fraction from within groups as young as these.

If a breeder wishes always to have official classifications on most of his milking cows, he will need to classify oftener than once every 2 years anyhow, since about 20 to 30% of his cows in milk will be heifers in their first lactations and nearly that many more will have first freshened between 1 and 2 years earlier.

Such considerations, rather than the increased accuracy of average ratings, will usually dominate the decision about how frequently to classify.

What corresponds to E in the official system would be about 1.4 to 1.7 times as large as E is here because the official system provides only six classes and, of course, few animals are put in the two extreme ones. However, keeping the six original classes but also designating three subdivisions of each, as was done here, might conceivably introduce psychological changes, such as an unconscious tendency to bunch the ratings closer to the middle of the range if there were thus 18 possible classes.

Extrapolating any of these findings to showring events seems risky. In the first place, the standards are different. Condition, fitting, etc., receive more attention in the showring. In the second place, cattle that reach the showring are already a highly selected sample. That is, the C component has been much reduced by leaving the poor and mediocre ones at home. The CJ component could be relatively larger in show ring placing if the C component were vastly reduced, although in general one would expect the CJ component to decrease as the C component did, although not necessarily in proportion to it. In the third place, emphasis on current condition and fitting might magnify the CY component in comparisons between placings in different shows, unless this is canceled by the showman's unwillingness to bring into the ring any animals in his show string that are temporarily out of condition.

Since the various experiments indicate so unanimously that disagreement between judges causes only a small part of the changes in type ratings and that dairy animals of all ages do change importantly in appearance from time to time, further work seems likely to lead to similar conclusions. Four stations, four of the five major dairy breeds, different intervals of time between classifications, and a wide range of ages have been involved. Different sets of judges with varying amounts of experience, some who have worked together closely, some who were chosen because of their dissimilarity of experience, produced very similar results. It is questionable whether further research of this kind will yield much new information. Still, perhaps a way could be found to divide further the residual variation and to describe more completely the changes in appearance. Also, the relations between the classification of parts (the "breakdown") and the over-all classification are little explored.

SUMMARY

Holsteins in the Iowa State College herd were classified three times at yearly intervals by three classifiers, working simultaneously and independently, to measure the amount and causes of change in type ratings of dairy females.

Cows change importantly in type from time to time, but disagreement in ideals between experienced classifiers is small. The largest changes in type occur between a rating made prior to first calving and another made sometime after calving.

Differences between heifers are smaller than differences among cows, or else the judges are less certain of the differences before first calving.

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CALCIUM METABOLISM WITH SPECIAL REFERENCE TO PARTURIENT PARESIS (MILK FEVER) IN DAIRY CATTLE: A REVIEW

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Parturient paresis, or milk fever, is a metabolic disease associated with parturition and the initiation of lactation. It is characterized by low total serum calcium and low or normal serum inorganic phosphorus levels. Outward symptoms are characteristic: In the early stages there may be generalized tetany; in later stages, paralysis and coma. An immediate favorable response usually occurs when the blood calcium level is raised either by the intravenous administration of calcium salts or by the inflation of the udder with air, thus inhibiting milk secretion. The condition occurs most commonly in aged, high-producing Jersey cattle. It is not prevalent at the second lactation and is seldom, if ever, observed in first calf heifers. There are breed differences in susceptibility, Jerseys and Guernseys being more afflicted than Holsteins. High milk production may be a predisposing factor, since reference to this condition first appeared in the literature with the development of high production in the dairy cow.

Before reviewing recent work bearing directly upon parturient paresis, a general survey of the information available concerning some of the aspects of calcium metabolism will be presented to provide a background of information as a basis for discussion of possible methods for the prevention of parturient paresis. The authors are of the opinion that this condition is a failure of the normal homeostatic mechanisms (principally the parathyroid glands) to maintain a normal level of blood calcium in the face of the great loss of calcium from the blood to the milk at the initiation of lactation in the high-producing dairy cow. In order to understand the conditions giving rise to milk fever one must understand these normal physiological mechanisms which become overtaxed.

SOURCES, UTILIZATION, AND EXCRETORY ROUTES OF BLOOD CALCIUM

The normal blood calcium level of cattle is approximately 10 mg. % *(86)*. The inorganic phosphate of the blood is nearly all completely diffusible between the blood plasma and the extravascular tissue fluids *(126)*. On the other hand, about 30 *(126)* to 50% *(242)* of the total serum calcium does not readily pass from the blood. Although there is evidence that a small fraction may be found in the form of a soluble complex of calcium phosphate *(168)*, the great majority of this nondiffusible calcium is bound to serum proteins *(13, 149, 157, 242)*. The diffusible calcium is mostly in ionic form. An equilibrium exists between the ionic and protein-bound fractions of the serum calcium, and the redistribution of calcium between these two states is a rapid process *(3, 216)*. The relationship between ionic calcium and serum protein is relatively constant, making it pos-

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sible to estimate the level of the former from the levels of serum protein and total calcium *(158)*.

The level of blood calcium is a reflection of the balance between that which enters and that which leaves the blood. The level is remarkably constant, and rather unphysiological experimental procedures must be undertaken in order to vary the level appreciably. Calcium enters the blood by absorption from the intestine, renal tubular reabsorption, and mobilization from the skeletal reserves. It leaves through the intestine to form metabolic or endogenous fecal calcium, through the urine, by deposition in the bone, and by utilization for growth, pregnancy, and lactation. Each of these sources of supply and loss will be considered in detail in following sections of this review.

The intestinal absorption of calcium. Although the distal portion participates to some extent, particularly when vitamin D is present in the diet *(103, 173)*, calcium apparently is absorbed primarily from the proximal portion of the small intestine, at least in rats. There is evidence that the caecum and large intestine may absorb some calcium in rats, but this pathway of absorption is far less important than is the small intestine *(250)*.

The calcium content of the diet per se will of course determine the amount of calcium absorbed from the gastro-intestinal tract, and in most species the blood calcium level tends to rise or fall with the dietary calcium level. For example, in sheep *(66)* and in rats *(23, 77, 210, 237)*, low calcium diets result in a marked decrease in the serum calcium level. In cattle, however, it is difficult to lower the blood calcium level with low calcium diets *(82, 159)*. This may be due in part to the difficulties encountered in attempting to devise low calcium diets for cattle because of the relatively high calcium content of roughages. It has been reported that high calcium diets or those containing a high Ca:P ratio will cause a slight increase of the blood calcium level of cattle *(201)*.

The efficiency of calcium absorption may be increased by two means: *(a)* increasing the demand of the animal for calcium as during growth or lactation and *(b)* decreasing the calcium intake. In goats and cattle, the highest level of "true digestibility" of orally administered Ca-45 is reached during the peak of the lactation period *(246, 248)*. Huffman *et al. (127)* reported a tendency of heavy milking cows to use dietary calcium more efficiently during the period of highest production. Total calcium absorption and retention is increased in rats with low body stores of calcium produced by previously feeding low calcium diets *(30, 58, 97, 171)*. The age of the animal determines the efficiency of calcium absorption. This is probably the result of the greater requirement of the young growing animal for calcium. Calcium absorption decreases with increasing age in rats *(30, 94, 111, 112)* and in cattle *(96)*. The greater efficiency of calcium absorption in the young animal could be a partial explanation for the low incidence of milk fever in young cattle.

In both rats and cattle, the lower the calcium intake, the greater the efficiency of calcium absorption *(94, 127)*. Animals will adapt to low calcium diets by increasing the efficiency of calcium absorption *(175, 176, 249)*; however, before such adaptation occurs, there must be a substantial loss of the body stores of

calcium *(175)*. Vitamin D may be involved in the mechanism of adaptation, at least in poultry. Chicks will not adapt to low calcium diets with increased intestinal absorption unless vitamin D is present in the diet *(163)*.

Another aspect of the influence of the metabolic requirement for calcium on calcium intake and thus indirectly upon absorption is voluntary self-selection of the diet. Thus, rats on a calcium-deficient diet show a preferential appetite for diets containing calcium *(205)*. Parathyroidectomized rats on a low-calcium diet voluntarily select solutions containing calcium over distilled water *(195, 196, 254)*. Increasing the dietary calcium, administration of vitamin D, or replacement therapy with parathyroid tissue will return the calcium appetite to normal *(195, 196)*.

The formation of relatively insoluble calcium complexes in the intestine tends to limit calcium absorption. Oxalates and phytates are the two most important substances in this respect. Oxalates are known to decrease the absorption of calcium in the rat because of the formation of relatively insoluble calcium oxalate, much of which is excreted as such *(226)*. In adult rats, particularly, the utilization of calcium from calcium oxalate is especially low *(146)*. Oxalates do not play an important role in calcium absorption in ruminants because they are broken down to bicarbonates and carbonates in the rumen. The formation of these compounds may result, however, in alkalosis, thus upsetting mineral equilibrium and affecting calcium metabolism indirectly *(226)*. It has been reported, nonetheless, that oxalates may decrease calcium absorption in ruminants if high levels of potassium are also ingested *(225)*. Phytates, present in rather high levels in cereals, are of considerable importance in calcium metabolism in monogastric animals because they compete for calcium in the intestine and thus limit the amount available for absorption *(121, 122, 123, 152, 161, 249)*. An enzyme, phytase, has been prepared from the intestinal tract of rats *(188)* and chicks *(218)*. Vitamin D tends to increase the phytase activity of rats and chicks fed with high-cereal rachitogenic diets and those fed noncereal, nonrachitogenic rations *(218)*. Phytates, however, do not appear to be an important factor in calcium absorption in ruminants. In sheep, ingested phytates are completely hydrolyzed in the gastro-intestinal tract, primarily in the rumen *(194)*.

The majority of evidence indicates that an increase in the acidity of the gastro-intestinal tract favors calcium absorption *(73, 108)*. However, the over-all retention of calcium may be unaffected since the oral administration of acid, although it may increase intestinal calcium absorption, also increases the urinary excretion of calcium *(72, 108)*. There is some evidence that increasing the acidity of the gastro-intestinal tract may be beneficial in converting a rachitogenic diet to a nonrachitogenic diet in rats *(210)*.

A few have reported that the Ca:P ratio of the diet is not an important factor in determining the amount of calcium absorbed. Thus, Axelsson and Eriksson *(5)* found in adult sheep that Ca absorption was directly correlated with the calcium content of the diet and that the amount of dietary P did not influence significantly the absorption of calcium. Patton *et al. (187)* reported a similar finding in young women, namely, that calcium retention was more

closely dependent upon the absolute level of calcium intake than upon the Ca:P ratio. The majority of evidence, however, indicates that the Ca:P ratio does play a role in determining the absorption of calcium. Thus, in growing calves *(180)* and in cattle *(238)* the level of dietary calcium or phosphorus tends to limit the absorption of the remaining element. In rats *(26, 210)*, sheep *(57)*, and goats *(83)* both the dietary levels of calcium and phosphorus and the Ca:P ratio are important in the production of rickets. There is good evidence that the requirement for vitamin D increases as the Ca:P ratio becomes wider in rats *(14)*. It is assumed that the formation of insoluble calcium phosphate in the digestive tract limits the availability of calcium for absorption. In this connection, Cremer *et al. (41)* found that vitamin D will increase the absorption of calcium only from insoluble calcium salts, such as calcium phosphate, and has no appreciable effect upon calcium absorption from soluble salts, such as calcium lactate.

Magnesium and manganese, particularly the former, have much the same influence upon calcium absorption as does the dietary Ca:P ratio. Magnesium sulfate supplementation of the diet of cattle decreases calcium absorption, especially if the dietary phosphorus is also low *(87, 183)*. The addition of manganese to the diet of cattle reduces calcium retention *(69, 193)*. In the early stages of magnesium deficiency in dogs there is an increase in calcium retention, presumably due to removal of Ca:Mg antagonism. Thereafter calcium absorption falls, resulting probably from nutritional breakdown *(139)*.

There is some evidence that an increase in the dietary protein will increase the calcium utilization *(76, 124)*, the increase not being entirely accounted for by the increase in growth rate of the rats used in this investigation *(76)*. Although this increase of calcium utilization occurred at both a low calcium and a moderate calcium intake, the protein effect was greater on the latter diet *(76)*. McCance *et al. (153)* reported similar findings with human subjects. Visek *et al. (248)* could find no influence of dietary protein on Ca absorption in dairy cattle.

Although Hart *et al. (108)* could find no consistent or favorable influence of glucose supplementation upon the calcium assimilation of dairy cows receiving a standard ration of alfalfa hay, corn silage, cereal grains, and concentrates, lactose seems to be of some importance in calcium absorption; supplementation of the diet with this sugar increases calcium absorption in calves *(198)*, young boys *(164)*, and lactating rats *(64)*. It has long been known that diets rich in lactose tend to prevent tetany and prolong the lives of parathyroidectomized dogs *(45, 46)*. This is true only if the diet contains, in addition to lactose, relatively high levels of calcium *(81)*.

Moderate amounts of dietary fat apparently favor *(25, 132)*, and larger amounts depress, calcium absorption *(67)* probably because of the formation of insoluble calcium soaps. It has been proposed that the favorable influence of fat is due to the formation of a readily absorbable bile-fattyacid-Ca complex *(67)*. The action of fat upon calcium absorption also seems to depend upon the calcium and phosphorus content of the ration, since supplemental fat is more effective in increasing the utilization of calcium from diets containing optimum amounts of phosphorus as compared to low P diets *(27, 132)*. In rats, the utiliza-

tion of calcium from calcium soaps may be dependent upon the demand for calcium and upon the presence of vitamin D *(172)*. It has been reported that the degree of saturation of the soap is *(12)*, and is not *(172)*, important with regard to the absorption of calcium from soaps. In adult humans, the fat content of the ration appeared to have little or no influence upon the absorption of calcium *(68, 219)*.

Vitamin D, with the exception of the dietary calcium intake, is the most important factor in regulating the absorption of calcium *(174)*. Experiments in the early 1920's *(107, 156)* indicated that a dietary factor present in green feeds and cod liver oil, which was distinct from the antiscorbutic factor and fat-soluble A, would increase calcium retention in rats and goats. Exposure of dry and lactating goats *(105)* or lactating cows *(106)* to artificial ultraviolet light or sunlight would also increase calcium retention. It is now generally accepted that vitamin D has a direct effect upon the mineralization of bone, as well as upon the intestinal absorption of calcium *(75)*. The influence of this vitamin on bone will be considered in another section of this review. Most evidence indicates that the action of vitamin D upon the intestine is confined to a direct effect upon calcium absorption, the decrease in the absorption of phosphorus observed in vitamin D deficiency being due to the precipitation of P by the increased levels of unabsorbed calcium *(170)*. There is evidence that vitamin D increases intestinal calcium absorption only under conditions in which the intestinal calcium is poorly soluble *(41, 103)*. Bile is required for the action of vitamin D *(74, 116)*. It has been proposed on the basis of studies with poultry that the action of vitamin D may be explained by its reaction with calcium at the intestinal absorptive barrier. Thus, the amount of calcium absorbed in the presence of excess vitamin D would be a function of the amount of vitamin D–calcium complex formed *(163)*.

Endogenous or metabolic fecal calcium. In rats *(250)* and cattle *(95)* the use of parenterally adminstered Ca-45 has indicated that calcium is excreted into the feces through all parts of the gastro-intestinal tract, but the small intestine is by far the most important excretory pathway. In cattle, the amount of endogenous fecal calcium is directly proportional to body weight and is little influenced by short-term dietary changes *(38, 247)*. After correcting for increase in body size, there is some indication that endogenous excretion increases slightly with age *(96)*. When cattle are fed low-calcium diets for prolonged periods, there is a decrease in the excretion of endogenous fecal calcium *(38)*. There is evidence that the amount of endogenous fecal calcium is influenced by vitamin D. Thus, in rats on a calcium- and phosphorus-free diet, vitamin D lack increased the excretion of metabolic fecal calcium *(169)*. The amount of unabsorbable calcium in the intestine may also determine the extent of metabolic calcium excretion. Thus, ingestion of high oxalate diets and the administration of chelates or calcium phosphate to rabbits increased the excretion of metabolic fecal calcium *(232)*. The supposition is that an unabsorbable calcium reservoir in the intestine competes for blood calcium across the intestinal wall.

Urinary excretion of calcium. In normal dogs over 99% of the calcium filtered through the glomeruli of the kidney is reabsorbed by the renal tubules *(168)*. In rats 90-97% is reabsorbed *(39)*. It appears that the renal clearance of calcium is dependent upon the ability of the renal tubules to reabsorb ionic calcium and upon the concentration of ionic calcium in the serum *(168)*. In general, the extent of calcium excretion in the urine depends upon endogenous (hormonal) factors which regulate tubular reabsorption and the serum calcium level and upon the dietary calcium intake per unit body weight *(136, 175)*. Sex, age, and dietary factors other than calcium, with the exception of ingested acids, have only minor effects upon urinary calcium excretion *(136)*. Ingestion of mineral acids by human subjects *(136)* and cattle *(108)* increases urinary calcium excretion.

Utilization of calcium for growth, pregnancy and lactation. Pregnancy and particularly lactation tend to lower the level of blood calcium as a result of the transfer of blood calcium to the fetal skeleton and to the milk. For example, Niedermeier *et al. (177)* have demonstrated a greater, more consistent decline in the serum calcium level of intact cows at parturition (initiation of lactation) than in mastectomized cows. Under normal conditions, however, blood calcium does not fall appreciably with pregnancy and lactation *(244)* except for a transient fall of the level of blood calcium with the initiation of lactation *(15, 71, 151, 177)*. This is true even though lactating cows are in negative calcium balance during at least the first part of the lactation period *(63, 251)*. The fall occurs in both the diffusible fraction and in total blood calcium *(151)*. However, if another calcium stress, such as low calcium diets or parathyroidectomy, is imposed upon the stress of pregnancy or lactation, there may be a marked fall in blood calcium *(24, 66)* and an increase in maternal and fetal mortality *(21)*. This is not always the situation. Duckworth and Hill *(47)* fed low-calcium diets to ewes throughout pregnancy and lactation with no reduction of reproductive performance, milk production, or lamb survival. Although pregnancy does not induce marked mobilization of skeletal calcium reserves *(24, 253)* lactation does *(65)*, particularly on low calcium diets *(24, 47)*.

Equilibrium between calcium of the blood and bone. Calcium transfers readily between the blood and the bone, the direction of transfer depending upon the current demand of the soft tissues for calcium. A large amount of calcium can be removed from the blood within a short time without resulting in an appreciable fall of the level of blood calcium because of the rapid mobilization of skeletal calcium *(109, 110)*. Actually, bone resorption and deposition occur in all bone constantly, although both processes are more rapid in regions of active growth. Decalcification occurs when the rate of resorption exceeds the rate of deposition *(131)*. The skeleton is a calcium reserve which releases calcium to the blood when it is needed and stores it avidly when the availability of calcium from intestinal absorption exceeds the requirements of the soft tissues *(10)*. Thus, in rats and cattle approximately 85% of an intravenous dose of Ca-45 or of the Ca-45 absorbed from the intestine is deposited in the bone *(93)*.

The skeletal calcium consists of two components on the basis of the rate of exchange between the calcium of the blood and that of the bone *(212, 213, 215)*. One fraction, the "labile" fraction, exchanges rapidly with blood calcium and is believed to consist of calcium located on the surface of the microcrystals of the bone salt *(69, 215)*. It has been reported that not all of the crystal surfaces are involved in this rapid exchange. Engfeldt and coworkers *(52, 54)* have demonstrated that only the young Haversian systems have a high rate of uptake, the older systems picking up the isotope slowly. It may be that the young areas take up isotope more readily because of the smaller size of the bone crystals in the newly formed areas *(54)*. The other component of the bone calcium, the "stable" component, exchanges slowly with blood calcium and may consist of calcium within the interior of the bone crystal where exchange occurs by the recrystallization of bone salt *(215)*. It has been reported that the crystallization and recrystallization processes which remove calcium from the exchangeable, that is labile, component to the stable component of the bone calcium occurs at a rapid rate. Thus, one hour after the administration of Ca-45 into rabbits one-half of the isotope fixed in the bone is no longer exchangeable by the usual in vitro techniques *(4)*. This may account, at least in part, for the failure of Carlsson *(31)* to find a "labile" fraction in the rat incisor.

The size of the labile component varies with age, the size being greater in the young animal *(40, 102)*. In fact, nearly all of the skeletal calcium of young rats exchanges rapidly with that of the blood *(102)*. If this situation is true in cattle, this may be another reason for the increase in the incidence of parturient paresis with age in cattle. Estimations of the size of the labile component in older animals vary from 15 to 33% of the total skeletal calcium *(40, 43, 59, 120, 235)*. There is good evidence that different bones have different rates of exchange, depending upon their anatomical location *(219)*. In fact, different areas of the same bone have different rates of exchange. Thus, in rats epiphyseal bone exchanges more rapidly than does diaphyseal bone *(2, 101, 102)*.

FACTORS REGULATING THE LEVEL OF BLOOD CALCIUM

Although some of the information available relating to the sources of calcium supply and loss and the pathways of calcium metabolism has been reviewed, little mention has been made of the regulators which act to maintain a constant level of blood calcium in the face of wide fluctuations in calcium supply and demand.

Influence of vitamins upon calcium metabolism. Although vitamin A is reported to have an influence upon bone metabolism *(8, 129)*, possibly regulating the gross shape and structure of bone *(160)*, vitamin D is by far the most important nutritional factor in regulating calcium metabolism, with the exception of calcium itself. As mentioned above, vitamin D has two effects upon calcium metabolism. It regulates intestinal absorption and it has a direct influence upon the bone. Thus, it apparently is involved in regulating the pathway between skeletal calcium and blood calcium. It is this latter function with which we are concerned in the present discussion.

A large number of investigators have demonstrated that vitamin D will cause bone resorption. Barnicot *(8)* found that crystals of vitamin D, but not cholesterol, will cause local resorption of bone when grafted to bone. In rats fed low-calcium diets vitamin D will induce bone resorption *(99)*. Vitamin D administration will induce hypercalcemia in dogs *(114, 133)* and rats *(208, 227)* fed low Ca or calcium-free diets to minimize the influence of the vitamin upon intestinal calcium absorption. Massive doses of vitamin D will also cause increased blood calcium levels in cattle *(48, 115, 118, 141)*. In this case, increased intestinal absorption of calcium as well as mobilization from the bones could account for the increase in blood calcium.

Vitamin D will protect parathyroidectomized animals against tetany by tending to maintain the blood calcium at normal levels even when such animals receive a calcium-free diet *(133)*. This vitamin, especially dihydrotachysterol, has been used successfully in the treatment of parathyroid insufficiency in humans *(200)*. Dihydrotachysterol is effective in this respect even when such patients are receiving a low-calcium diet *(199)*. It will increase the blood calcium level to a much greater extent than would be predicted by its anti-rachitic properties *(155)*. Actually, any of the compounds having vitamin D activity are more effective in the treatment of parathyroid insufficiency than is parathyroid extract itself *(154)*. Parathyroidectomized dogs receiving vitamin D in amounts sufficient to maintain normal levels of blood calcium and phosphorus can complete a normal life cycle, including reproduction and lactation *(137)*.

Carlsson *(32)* observed that vitamin D increased the Ca-45 uptake by bone but did not increase the bone ash weight of rats on a low calcium diet. This type of diet was used in order to minimize the influence of the vitamin upon intestinal calcium absorption. From these results it was concluded that vitamin D favors bone resorption. This conclusion was further substantiated by the fact that the blood calcium level remained normal in spite of the low calcium diet in the vitamin D–treated animals. It appears that the hypocalcemia in vitamin D–deficient rats may be due to an inability to utilize stored bone salt even when such stores are filled by previous diets adequate in calcium and phosphorus. In such vitamin D–deficient rats, the blood calcium falls within 24 hours after the rats are placed on a low calcium diet *(33)*. Singer and Armstrong *(214)* reported that vitamin D does not alter the turnover rate of the "stable" fraction of bone calcium. It would appear that vitamin D functions primarily in intestinal absorption but that it also is involved in maintaining a normal blood calcium level by skeletal mobilization of calcium when calcium availability is limited.

Influence of hormones upon calcium metabolism. There is evidence that the administration of thyroid hormone will increase the excretion of fecal calcium in intact and hypophysectomized rats *(192)*, in dogs *(143)*, and lactating dairy cattle *(181)*. There is no effect upon the serum calcium or phosphorus levels of cattle *(181)*, dogs *(143, 150)*, or guinea pigs *(178)*. Engfeldt and Hjertquist *(53, 55)*, however, have reported that thyroxin and pituitary thyrotrophic hormone, in the presence of an intact thyroid, will increase serum phosphorus and decrease the serum calcium, thus resulting indirectly in parathyroid stimulation

in the rat. Thyroxin has little or no influence upon urinary calcium excretion *(181, 192)* and any increase which may occur is due to general diuresis with no change in the concentration of urinary calcium *(150)*. There is good indication that prolonged administration of thyroid hormone *(178)* or excess endogenous production of thyroid hormone *(62, 178)* may result in excessive bone destruction.

Cortisone administration to growing rats decreases the normal osteolytic activity at the ends of the long bones to such an extent that a dense zone of calcified cartilage matrix is formed *(60)*. This is unique with the rat and does not occur in mice, guinea pigs, or rabbits *(61)*. It has been reported that cortisone administration to nephrectomized dogs increases serum calcium to high levels along with a rise in serum magnesium and phosphorus *(84)*. Ulrich *et al. (241)* reported that the prolonged injection of ACTH causes a slight increase in urinary and fecal Ca-45 excretion in both hypophysectomized and intact rats but has little effect on the skeletal uptake. Hypophysectomy alone decreased the skeletal uptake of Ca-45.

Krishnan *(138)* reported that daily injection of "Antuitrin" growth hormone preparation increased calcium retention and decreased fecal calcium excretion but had no effect upon urinary calcium excretion in guinea pigs and rats. Ulrich *et al. (240)* found that "pure" growth hormone increased the tibial uptake of Ca-45 in hypophysectomized rats as compared to hypophysectomized controls but no change in calcium content of bone ash occurred. In acromegaly there was an increase in the urinary calcium and phosphorus excretion, although the blood levels remained normal *(9)*. Engfeldt *(51)* reported that the administration of growth hormone to hypophysectomized rats increased the blood phosphorus level, thus stimulating the parathyroid activity indirectly. Administration of growth hormone to hypophysectomized-parathyroidectomized rats also increases blood phosphorus; thus, the increase in blood phosphorus was not due to the parathyroid hormone. Hertz and Kranes *(113)* reported that parathyroid hypertrophy and hyperplasia occurred in rabbits with the injection of anterior pituitary extract. Törnblom *(236)* found that the pituitary has no direct influence upon the parathyroids but does increase blood phosphorus and tends to decrease blood calcium. It is suggested that the increase in blood phosphorus is the stimulus to the parathyroids. Some evidence is presented indicating both growth hormone and ACTH are responsible for the increase in blood phosphorus, probably by way of their influence upon carbohydrate metabolism.

The influence of estrogen on calcium metabolism in birds has recently been reviewed by Lorenz *(144)*. Although estrogens have no influence on the level of blood calcium in mammals *(140)*, they have an effect upon the skeletal system, the nature of the influence depending upon the species. Urist *et al. (243)* have found that estrogens stimulate endosteal bone formation and inhibit bone resorption in mice. In the young growing rat endosteal bone is not produced but endochondral bone formation is interfered with because of the inhibition of the normal resorptive processes involving the cartilage matrix and new bone. Estrogens have no specific effect on guinea pigs, rabbits, cats, or dogs although proliferation of the epiphyseal cartilage and new bone formation may be depressed.

Vitamin D does not play a role in the mechanism of estrogen-induced endosteal bone formation in mice *(206)*.

The hormone secreted by the parathyroid glands is one of the most important factors regulating the level of blood calcium. Salvensen *(202)* concluded that the symptoms of parathyroid removal were due to calcium deficiency and that the parathyroids normally regulate the level of blood calcium on the basis of observations that parathyroidectomized dogs would survive if fed high calcium diets and that the intravenous administration of calcium salts would cure and prevent the tetany resulting from this operation. In a series of experiments, Collip *(37)* demonstrated that extracts of the parathyroids would increase the blood calcium level of intact and parathyroidectomized dogs. Greenwald and Gross *(80)* found that similar extracts resulted in the excessive destruction of bone, increased the serum calcium level, and increased urinary phosphate excretion. Hastings and Huggins *(110)* repeatedly removed, decalcified, and transfused the decalcified blood into dogs and found that under such conditions the blood calcium level was maintained more readily in intact than in parathyroidectomized dogs. From these results it appears that although skeletal mobilization of calcium can occur to some extent in the absence of the parathyroids, parathyroid hormone is important for such mobilization. Observations by Bodansky and Duff *(22)* have also suggested an inability of the parathyroidectomized animal to mobilize skeletal calcium. They observed a failure of the pregnant, parathyroidectomized rat to maintain a normal serum calcium level. At the conclusion of pregnancy the long bones of the parathyroidectomized animals weighed more, in relation to the pregestational body weight, than those of the intact controls. The per cent ash of the bone was also higher in the former group, whereas the calcium/phosphorus ratios were nearly identical. Parathyroid hormone has no influence upon the intestinal absorption of calcium *(220)*.

It has been known from the early work of Greenwald *(78)* and Greenwald and Gross *(79)* that parathyroid removal results in marked phosphate retention by the kidney. These early findings have been confirmed and extended by the work of Talmage and Kraintz *(228, 229)*, who have demonstrated an immediate decrease in urinary phosphate excretion after parathyroidectomy in the rat. The administration of parathyroid extract results in an increase in phosphate excretion. This increase is due, at least in part, to a decrease in the renal tubular reabsorption of phosphate *(29, 100, 104, 135)*. An increase in the glomerular filtration rate may *(135)* or may not *(29)* be partially responsible for the increase in urinary phosphate excretion. These differing results may be due to the particular extract employed. Both the nature of the extract and the route of administration are important to the manner in which parathyroid extracts cause increased urinary phosphate excretion. Handler and coworkers *(90, 91, 92)* have observed that the intravenous administration of parathyroid extract will increase the urinary phosphate excretion of dogs. This increase is associated with increased renal blood flow and glomerular filtration rate, which are secondary to an increase in systemic blood pressure. The subcutaneous administration of similar extracts, on the other hand, increases phosphate excretion by

reducing the tubular reabsorption of phosphate without changing the glomerular filtration rate. In the latter instance the serum calcium increased to high levels. Chemical fractionation of the extract produced two components: one which would increase glomerular filtration rate, the other which increased the blood calcium level and reduced tubular phosphate reabsorption. Stewart and Bowen *(222)* have confirmed previous findings that the intravenous administration of parathyroid extract will induce an immediate increase in renal phosphate excretion. They believe this to be an artifact since similar activity could be demonstrated in extracts inactivated, as regards their ability to raise serum calcium, by formaldehyde treatment. Further, similar activity could be demonstrated in extracts of spleen and kidney prepared in the same manner as parathyroid extract. Davies and Gordon *(42)* have also prepared a fraction from parathyroid extract that will increase phosphate excretion without increasing the serum calcium level. Since glomerular filtration rates were not measured in these last two studies, it cannot be determined if the factor causing increased urinary phosphate excretion is similar to that prepared by Handler and coworkers. The extract of the latter increases the glomerular filtration rate without increasing tubular reabsorption.

The early work with the parathyroids led to two conflicting opinions regarding the actual mechanism by which these glands increase the level of blood calcium. One school of thought *(1)* supported the view that ". . . the parathyroid hormone in some way affects the phosphate dissolved in body fluids in such a way as to make it more readily excreted by the kidney with a resulting decrease in the serum phosphorus values; resorption of the calcium-phosphate salt from the bone-resorbing surfaces is thereby increased; there results an elevated serum calcium level together with the depressed serum phosphorus level. Once this new state of equilibrium has been reached there would be no further changes if it were not for the fact that the higher serum calcium level leads to an increased calcium excretion in the urine; this loss of calcium in the urine is a factor tending to cause undersaturation of the body fluids again so that unless there is a supply of calcium from the gastro-intestinal tract the bones will have to supply the deficit." Thus, the primary action of parathyroid hormone, in the opinion of this group, is an increase in the renal excretion of phosphorus, the other changes such as the increase in blood calcium being secondary effects. The other school *(233)* proposed that the primary action of the hormone was to induce dissolution of bone, possibly by increasing the numbers and activity of the osteoclasts of bone.

At the present time there is good evidence that endogenously produced parathyroid hormone and certainly parathyroid extracts have direct effects on both the renal excretory mechanisms and the bone. It is known that the increase of phosphate excretion, which occurs with parathyroid extract administration, precedes the rise of blood calcium *(1)*. As mentioned above, such extracts result in an active decrease in the tubular reabsorption of phosphate. Schaaf and Kyle *(204)* have demonstrated that in human hyperparathyroidism with attendant hypercalcemia the renal tubular phosphate reabsorption is low. If a similar

hypercalcemia is induced in normal controls by the intravenous administration of calcium salts, however, phosphate reabsorption does not change unless parathyroid extract is also administered, in which case it decreases. Thus, something is present in the hyperparathyroid which actively reduces phosphate reabsorption irrespective of hypercalcemia. Milne *(165)* has reported a low urinary phosphate clearance in a hypoparathyroid patient which returned to normal upon parathyroid extract administration. Similar injection into normal people had no effect upon phosphate clearance, and it was suggested that the primary action of the extract in the hypoparathyroid individual is a renal one, whereas under normal conditions the hormone stimulates osteoclastic bone resorption.

Talmage and Kraintz *(228)* have found that the renal excretion of injected P32 is greatly reduced within one hour after parathyroidectomy in rats. In an extension of these studies, Talmage and Kraintz *(229)* reported an immediate increase in the renal Ca-45 excretion along with the reduced phosphate excretion. The excretory rates returned to normal within 24 hours in spite of the continued high blood phosphorus and low blood calcium levels. The same picture in reverse occurred in normal rats injected with parathyroid extract, except that the excretory rate of phosphate remained above normal. From these results the authors concluded that the parathyroids have a direct effect on the renal excretion of both calcium and phosphorus. The return of the excretory rates of calcium to normal is explained as a change in the renal threshold for calcium. When serum calcium becomes adjusted to the new level, calcium excretion returns to normal. Excretory changes observed after the adjustment of the serum calcium and phosphorus levels to new thresholds are due to extra-renal changes which increase or decrease the entry of calcium and phosphorus into the serum. In an earlier paper, Talmage and coworkers *(231)* reported that parathyroid extracts remove significant amounts of previously deposited Ca-45 and P32 from the subepiphyseal plate region of the long bones of rats. The amount of phosphorus removed appeared greater than the amount of calcium removed. They assumed that the parathyroids act directly upon the bone but that this effect is enhanced by the additional effect upon the kidney, where phosphate excretion is increased since the serum P levels can be increased above normal by extending hormone treatment in spite of the greatly elevated renal phosphate excretion, indicating mobilization of skeletal phosphate.

It has been definitely established that the parathyroids can have a direct influence upon bone resorption. Barnicot *(7)* and Chang *(36)* have found that local grafts of parathyroid tissues to bone result in local bone resorption, whereas similar grafts of other tissues, such as thyroid or adrenal, do not. Although early work indicated that the administration of parathyroid extract to nephrectomized animals would not result in increased serum calcium levels *(167, 239)*, later reports have demonstrated that this response will occur in the nephrectomized animal *(134, 221, 223, 230)* and that this response could still be elicited several weeks after nephrectomy when the animal is maintained by intraperitoneal lavage *(85)*. Nephrectomy alone will result in parathyroid stimulation and induce bone changes similar to those produced by the administration of parathyroid

extract *(128, 207)*. Parathyroid extracts produce the bone lesions typical of hyperparathyroidism in nephrectomized, as well as intact, rats *(128)*. Jacobs *(130)* has found that preventing the drop of blood phosphorus, which usually occurs with parathyroid extract administration, by the intravenous administration of phosphate will still result in an increase of the blood calcium, indicating that extra-renal mechanisms regulate the serum calcium level in this case. Monahan and Freeman *(166)* came to a similar conclusion upon finding that the serum calcium level decreased 50% within 72 hours in nephrectomized, parathyroidectomized dogs but no similar decrease occurred with nephrectomy alone. In a different approach to the problem, Stewart and Bowen *(221)* found that the intravenous administration of oxalate lowered the serum calcium level to about 6 mg. %, the level returning to normal within 6 hours in intact, as well as nephrectomized, dogs. In parathyroidectomized dogs a similar decrease in this "oxalate tolerance" curve occurred but there was no return to normal within 6 hours, indicating that the parathyroids regulate blood calcium independently of the kidney. Recent evidence indicates that the parathyroids may destroy the organic matrix of bone, thus liberating bone salt indirectly. Carnes *(34)* found that bone matrix may be broken down by parathyroid extract irrespective of its mineral content. The resulting level of serum calcium and the severity of metastatic calcification that occurred were functions of the mineral content of the resorbed matrix. Engel *(49)* and Engel *et al. (50)* reported that parathyroid extract causes depolymerization and solution of the glyco-protein ground substance of bone and epiphyseal cartilage. There was an associated increase in the serum mucoprotein level, which was related to the degree of bone change and roughly to the amount of hormone administered. It was suggested that the effect of parathyroid extract is brought about by its influence on the state of the mucoprotein of bone and cartilage.

From results reported in the literature it is difficult to assess the importance of the parathyroids in calcium metabolism of ruminants. Parathyroidectomy appears to cause a decrease in the serum calcium level of goats *(28)*. Robinson *et al. (197)* reported that the administration of rather high levels of parathyroid extract to calves resulted in a great increase in the serum calcium level. Hibbs *et al. (119)* found only a small increase of the blood calcium level of cows that received a single subcutaneous injection of 2,000 to 3,000 units of parathyroid extract. Lotz *et al. (145)* reported that the administration of 1,000 to 3,000 units of parathyroid extract to sheep resulted in the removal of previously deposited P32, but not of Ca-45, from the skeleton. The blood phosphorus level increased; the blood calcium level remained normal. There was a slight increase in the urinary phosphate excretion, which could be accounted for by the increase in the level of blood phosphorus. They concluded that in sheep the parathyroid extract acts directly upon bone to remove primarily phosphorus.

The primary stimulus to parathyroid secretion is the level of the serum calcium *(56, 185, 186)*, and factors that result in hypocalcemia will stimulate the parathyroids whereas those resulting in an increased blood calcium level will depress parathyroid activity. Pregnancy had been shown to result in increased

parathyroid size in rats *(179, 211)*, especially when superimposed on low calcium diets *(211)*. Pregnancy may not affect parathyroid size in rabbits and goats but lactation does *(28)*. Growth hormone *(51, 236)*, ACTH *(236)*, thyrotrophic hormone and thyroxine *(53, 55)* and anterior pituitary extracts *(17, 113)* increase parathyroid activity. Nephrectomy, as well as urethral ligation will increase parathyroid activity *(6, 179, 190)* as will renal disease in humans *(184)*. The intravenous injection of calcium salt into dogs and rabbits will cause parathyroid depression; the administration of phosphate causes hyperplasia *(189)*. In fact, if the ingestion of high levels of phosphate salts is continued for prolonged periods, bone changes characteristic of hyperparathyroidism may occur *(203)*. Low-calcium diets increase parathyroid activity whereas high-calcium diets decrease the size and activity of these glands in rats *(28, 35, 44, 56, 89, 98, 147, 203, 211, 224, 234)*, rabbits *(11)*, birds *(148)*, swine *(142)*, and sheep *(18)*.

MILK FEVER

Review of literature. In view of the excellent review of parturient paresis in dairy cattle by Hibbs *(117)* a detailed survey of the literature in this field is not warranted. Some of the pertinent information published subsequent to this review will be discussed.

A number of reports have appeared indicating an apparent association of adrenal cortical activity with milk fever. A decrease of serum citric acid *(16, 252)* and higher serum pyruvic and lactic acid levels *(252)* have been reported in cows exhibiting symptoms of milk fever in comparison with normally calving controls. It has been suggested that a failure of the glycolytic cycle occurs in milk fever *(252)*. Van Soest and Blosser *(245)* found a marked increase of blood glucose and pyruvic acid in both normally calving cows and in cows developing milk fever, the levels being considerably higher in the latter group. Statistically significant negative correlations between blood glucose and plasma phosphate and between blood pyruvate and phosphate were observed in the cows with milk fever. The correlation between blood glucose and pyruvate was positive and highly significant in both normally calving and milk fever cows at parturition. The authors suggest that these changes may be associated with an increased output of adrenal hormones. In this connection, Garm *(70)* has reported marked lymphopenia and eosinopenia as well as adrenal cortical hypertrophy in cows with milk fever and has suggested this condition is a "disease of adaptation" (Selye). Holcombe *(125)* has found that cows with milk fever show a low excretory level of urinary-reducing corticoids and neutral steroids, indicating adrenal cortical exhaustion. Since a decrease in the circulating lymphocytes and eosinophils and an increase in neutrophils and blood sugar occurs at parturition, Merrill and Smith *(162)* suggest that an increased secretion of adrenal cortical hormones occurs in response to the stress of parturition. The administration of ACTH has a similar effect to that of parturition on these blood constituents. In a continuation of this study *(217)* these workers report that milk fever produces sufficient stress to increase adrenal cortical activity as measured by the leucocyte response. In such cows adrenal cortical exhaustion was not evident since ACTH

administration resulted in the usual typical response. It would appear that the change of adrenal cortical activity associated with milk fever is not the cause but rather the effect of the stress imposed by this condition.

Ward *et al. (251)* have determined mineral balances in dairy cattle before and after parturition. They observed a severe negative calcium balance for about 15 days prepartum in three mature Jersey cattle that developed milk fever after parturition. The prepartal calcium balances were positive in mature Jerseys that did not develop milk fever and in first calf heifers. In all three groups calcium balances were negative after parturition. The authors suggest that milk fever may be preceded by a period of defective calcium absorption from the intestine or by excessive excretion of endogenous fecal calcium.

Owen *(182)* has studied the influence of complete milking at calving time on the incidence of milk fever. Ayrshire, Guernsey, and Jersey cows were divided into three groups at parturition. One group was partially milked for 3 days after calving. The second group was completely milked by conventional methods; in a third group the completeness of milking was aided by the administration of oxytocin 2 hours after calving. From the results, it was suggested that complete milking had no appreciable influence on the occurrence of milk fever. The incidence of this condition was so low in all three groups, however, that it is difficult to arrive at a definite conclusion in this respect.

In an interesting survey of parturient paresis, Hallgren *(88)* has studied 881 cases of "milk fever" in Sweden. Approximately 85% of the animals classified by field diagnosis as exhibiting symptoms of milk fever responded immediately to intravenous calcium therapy. Blood samples were collected from 77 of the animals that did not immediately respond and were analyzed for serum calcium, magnesium, phosphorus, and potassium. Only 12 of these cases were "normal" milk fever characterized by low serum calcium level. A second course of calcium therapy was effective in relieving the condition. Nine of the cases were "down" as a result of a severe primary heart condition; hypocalcemia was not manifest. A large number of the remaining animals were classified as "downers." In this condition the animal is alert but unable to rise. The serum calcium level may be low or normal and the serum phosphate level is low, often virtually nonexistent. Such animals respond favorably to intravenous phosphate administration. This report emphasizes the fact that a number of disorders may occur in high-producing cows coincident with parturition, which are often indistinguishable from "true" milk fever without a determination of the levels of serum calcium and phosphorus.

Prevention. The majority of evidence accumulated through the years concerning the etiology of parturient paresis strongly indicates that this condition results from a failure of homeostatic factors (principally the parathyroid glands) that normally operate to maintain the blood calcium at a constant level in the face of the drain of calcium to the milk with the initiation of lactation. On this basis the approach to milk fever prevention would involve either the preparation of these physiological mechanisms for optimal activity in advance of the demands of lactation or the temporary substitution of these mechanisms by artificial means

until they can become sufficiently active to meet the requirements of lactation by the adequate mobilization of calcium. Calcium lost from the blood can be replaced in only two ways, either by increasing the intestinal absorption of calcium or by increasing the mobilization of previously deposited skeletal calcium. Attempts to prevent milk fever by increasing the calcium content of the diet have been ineffective. This is probably because the extent of intestinal absorption cannot meet the lactation requirement of the high-producing cow, particularly after a period of low calcium demand (last portion of preceding lactation and the dry period) and in older animals when the efficiency of calcium absorption may be reduced. Thus, the better approach to milk fever prevention seems to be the stimulation of the mobilization of bone salt either by activating the normal endogenous factor (parathyroids) before lactation begins or by causing the mobilization of bone salt by artificial methods until the former mechanism can assert itself. The two methods of prevention, one proposed by Hibbs and Pounden *(118)*, the other by Boda and Cole *(20)*, which hold the most promise to date are based on these premises.

Hibbs and Pounden *(118)* have presented a summary of their work of the past several years on the influence of the prepartal oral administration of massive doses of vitamin D_2 (30 million units/day) on the incidence of milk fever. Their results indicate that milk fever can be largely prevented by this procedure. In cows receiving such treatment the usual fall of serum calcium and phosphorus at parturition is prevented, probably as a result of the skeletal mobilization of bone salt. Some increase in the intestinal absorption of these elements may also occur. These workers are of the opinion that the effect of the vitamin is to replace the calcium mobilizing effect of endogenous parathyroid hormone during the critical period at parturition. Previous attempts to reduce the incidence of milk fever by feeding smaller doses (1 to 5 million units of vitamin D_2 per day) for prolonged periods (2 to 4 weeks) were unsuccessful. This was attributed to an insufficient calcemic effect at parturition coupled with inhibition of parathyroid activity resulting from the prolonged prepartal elevation of the blood calcium level. The major disadvantage of this method is that the expected calving date must be rather accurately predicted so that the vitamin may be administered for at least 4 or 5 days but no longer than 7 days before parturition because of the possibility of hypervitaminosis with associated calcification of the soft tissues. Although these workers have used vitamin D_2 in their studies, it would be interesting to determine the dose of dihydrotachysterol required to prevent milk fever in view of its much more pronounced calcemic effect than either vitamins D_2 or D_3.

The second approach to milk fever prevention is that suggested by Boda and Cole *(19, 20)*, which is based upon the use of low-calcium, high-phosphorus prepartal diets in order to stimulate the production of endogenous parathyroid hormone by causing stimulation of these glands before the initiation of lactation and the increased demand for calcium mobilization occur. The evidence indicates that this procedure is effective in reducing the incidence of milk fever in high-producing Jersey cattle. The low-calcium prepartal diets are not detrimental

to milk production, parturition, or the health of the calf. This aspect has been discussed in detail by Boda in a recent paper *(19)*. The major disadvantage of this procedure is one of management imposed by the apparent necessity of feeding extremely low calcium rations. This requires a rather marked restriction of the intake of roughages, which are relatively high in calcium. Thus, the dry cows must be maintained in a dry lot and fed individually for the last month of the dry period in order to reduce the calcium intake and minimize the possibility of bloat, which might occur by group feeding these preventive rations which contain a relatively high proportion of concentrates to roughage. The rations that have been used to date have consisted of oat hay and either ground barley supplemented with mono-sodium phosphate (technical grade) or a basic concentrate mixture consisting of barley, wheat bran, cottonseed meal, and the phosphate salt. Daily intakes of hay varied from 5 to 8 lb.; concentrates from 8 to 12 lb. for a 1,000-lb. animal. It may be that the modification of the diets now in use for milk fever prevention, such as the addition of low-calcium grass silage or the incorporation of scabrous and bulky material to the ration, will minimize the basic management problem.

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SIRE BY HERD INTERACTION IN PRODUCTION TRAITS IN DAIRY CATTLE[1]

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The environment that should be provided breeding populations under selection has received much discussion *(6, 7, 12)*, but little evidence has been available upon which to base decisions or recommendations. Although many would argue that selection should be practiced in an environment comparable to the one in which the strain or breed is expected to perform, Hammond *(7)* has subscribed to the thesis that the environment provided should be the one that permits the maximum expression of the trait being selected. In addition to other considerations, Hammond's suggestion might be viewed in the light of possible hereditary-environmental interactions if the individuals are to perform in an environment differing greatly from the test environment. Falconer *(3)* has defined this problem where the choice of only two alternative environments is concerned, but the general problem defies such simple characterization and seemingly demands an examination of the actual situation for a large sample of genotypes and environments.

This problem of the appropriate environment may be important in connection with choosing sires for use in artificial insemination. The inability to appraise accurately the influence of general feeding and management conditions on a sire's proof probably accounts for most of the mistakes in selecting proved sires. Nevertheless, there is the possibility that a sire by herd interaction might contribute to the errors of appraising a proof where the proof is based on the performance of a bull's daughters in only one or a few herds.

Bulls selected for use in artificial insemination generally have come from the better managed dairy herds, and they have been selected on the basis of their progeny's performance under better than average conditions of feeding and management. However, their artificially sired daughters performed under a variety of environmental circumstances, which on the average are less favorable than those provided the daughters that were originally appraised in selecting the sire. The practical question arises as to whether sires selected on the basis of progeny performance in the better managed herds produce offspring that respond favorably under good conditions but that are unable to withstand satisfactorily the rigorous conditions to be experienced in practical dairy herds. Should such a

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circumstance exist, it could result from the interaction of the sire's genotype (a sample half of which is transmitted to each daughter) and the specific herd environmental circumstances under which the daughters perform. A second possible explanation might be the nicking of the sire's genotype with the genotype of the dams to which he is mated. Heizer *et al. (9)* indicated that nicking might be of some consequence, but Seath and Lush *(15)* and Johnson *et al. (11)* did not find evidence that it was an important source of confusion in evaluating the progeny test in dairy cattle.

The object of this study was to estimate the importance of sire by herd interaction effects for production traits as reflected by the magnitude of the sire by herd interaction variance. In nonstatistical terms the question asked is whether the relative ranking of a group of sires, based on their daughters' performance in the same herd, changes more than would be anticipated from chance and errors of appraisal when the production of other daughters of these same bulls is measured in other herds.

DATA AND METHODS

The data studied consisted of 305-day, 2×, mature equivalent D.H.I.A. lactation records of daughters of Guernsey, Holstein, and Jersey sires in service in artificial breeding associations throughout the United States since 1939 whose daughters had one or more records reported during the period 1946 to 1950. These data are summarized by breeds in Table 1.

TABLE 1
Summary of data by breeds

Breed	No. of daughters	No. of sires	No. of herds	No. of sire-herd classes	305-day 2×, M.E.		
					Milk	Test	Fat
					(lb.)	*(%)*	*(lb.)*
Guernsey	5,234	883	1,907	3,545	8,057	4.86	392
Holstein	17,581	1,260	5,359	12,305	12,185	3.60	439
Jersey	1,939	393	829	1,253	7,761	5.33	413

Animals included in the study were born the year the sire entered the stud or during subsequent years; hence, records making up the sire's original proof were not studied. Only the records initiated during the period 1946 to 1950 were included to minimize the possible influence of long-time trends and differences in production levels during the war and postwar periods.

When sires have daughters in only one herd, as is the situation with most sires proved in natural service, variance resulting from genetic differences among sires within herds and that resulting from specific sire by herd influences cannot be separated. In analyses on an intraherd basis whatever contribution is made by the herd-sire interaction is recovered as an addition to the sire component. However, where a group of sires are used in several herds, an estimate of the sire component of variance is possible as well as that ascribable to sire by herd interaction.

Since the major objective was to estimate the variance associated with possible sire by herd interaction effects, intra-cow variability was not computed and only one record was used for each cow to reduce the volume of computations. This record was chosen by machine, and the basis of choice was independent of the size of the record or the age of the cow at the time the lactation was initiated. Routine computations were handled by International Business Machines.

The analysis of variance made with Method I as described by Henderson *(10)* was used to separate the intra-state variance for each of the breeds into components resulting from herd, sire, sire by herd differences, and differences among paternal sisters in the same herd.

RESULTS

Inasmuch as the variance resulting from state to state differences could not be intelligently interpreted, and since many of the artificial breeding associations operate largely within one state, only the division of the intra-state variance is presented. Values for the components of variance associated with herd, sire, herd by sire differences, and differences among paternal half sisters in the same herd (residual) are given in Table 2. The corresponding percentage which each

TABLE 2

Components and per cent of intra-state variance by breeds

Source	Components			Percentages		
	Milk[a]	Test	Fat	Milk	Test	Fat
			Guernsey			
Herd	7,215	0.0319	1,833	25.1	14.0	29.3
Sire	1,910	0.0311	339	6.6	13.7	5.4
Sire-herd	−285	0.0014	−88	0.0	0.6	0.0
Residual[b]	19,600	0.1633	4,080	68.3	71.7	65.3
			Holstein			
Herd	17,966	0.0156	2,546	30.1	15.2	32.3
Sire	3,522	0.0121	533	5.9	11.8	6.8
Sire-herd	1,238	−0.0019	96	2.1	0.0	1.2
Residual[b]	36,998	0.0749	4,707	61.9	73.0	59.7
			Jersey			
Herd	9,044	0.0259	2,543	19.9	10.0	34.9
Sire	3,466	0.0310	459	7.6	11.9	6.3
Sire-herd	−13,243	0.0257	−230	0.0	9.9	0.0
Residual[b]	32,945	0.1772	4,285	72.5	68.2	58.8

[a] Components for milk expressed as hundreds (last two digits rounded).
[b] Among paternal sisters in the same herd.

component comprises of the intra-state variance is also presented. Striking differences in the residual variances for milk and fat percentage for the breeds studied made the appropriateness of pooling the information for all three breeds subject to question.

The sire by herd components for each of the traits for the individual breeds show much change in sign such as might be anticipated from fluctuation about a

population value of near zero. Individual breed values for test, with the exception of the Jerseys, indicate sire by herd effects for test to be inconsequential. In any case the more limited data leave the variance components for Jerseys with larger sampling errors than the estimates for the two other breeds. For Guernseys and Jerseys, sire by herd interaction variance estimates are negative for milk and fat and must be interpreted as zero or nearly so. For Holsteins, the sire by herd interaction component of variance for milk is approximately one-third the size of the component for sire differences. The same component for fat is something less than one-fifth the size of the component for sire differences.

Although even approximate methods are not available for computing sampling variances for components of variance obtained from nonorthogonal data by using the procedure followed in this study, the data for Holsteins were divided into 20 groups to provide some notion of the sampling variability. Ten divisions were made by sorting on the third digit of a seven-digit sire registration number. Each of these 10 groups was subdivided by including in one group the sires with 0 to 4 inclusive as the fourth digit of their registration number and the sires with 5 to 9 inclusive in a second group. This division of the data by sires markedly reduced the number of interaction degrees of freedom within state, but provided groups without systematic state or time biases in composition.

The data for each group are summarized in Table 3. Results of the analysis for the 20 groups for milk, fat per cent, and fat are given in Table 4. Sire by herd interaction components for each trait show an even distribution of plus

TABLE 3

Summary of the 20 groups of Holstein data

Group	No. of daughters	No. of sires[a]	No. of herds	No. of sire-herd classes	305-day 2× M.E.		
					Milk	Test	Fat
					(lb.)	*(%)*	*(lb.)*
1	563	44	408	419	12,072	3.61	435
2	887	31	534	552	12,091	3.60	436
3	525	29	357	388	12,234	3.58	438
4	981	40	655	735	12,214	3.56	435
5	926	47	589	641	12,341	3.65	452
6	1,030	55	715	767	12,263	3.64	447
7	889	41	582	613	12,149	3.62	440
8	1,187	49	778	830	11,768	3.64	428
9	1,007	38	644	692	12,515	3.58	457
10	988	54	594	675	12,096	3.64	433
11	655	28	387	417	12,078	3.64	441
12	905	54	590	647	12,378	3.59	445
13	641	24	435	445	12,240	3.60	441
14	775	34	490	518	12,444	3.62	451
15	624	35	434	448	12,121	3.62	433
16	1,377	53	803	933	12,291	3.55	436
17	1,112	55	718	760	12,004	3.60	432
18	716	31	485	505	11,931	3.58	427
19	616	40	426	449	12,202	3.55	433
20	1,177	55	782	870	12,247	3.60	441

[a] Represents the number of degrees of freedom for sires within states.

TABLE 4

Summary of herd, sire, and sire-herd interaction components of variance for Holstein data subdivided into 20 groups

	Milk[a]			Test			Fat		
Group	Herd	Sire	Sire-herd	Herd	Sire	Sire-herd	Herd	Sire	Sire-herd
1	4,600	1,887	10,063	0.0094	0.0050	0.0059	2.087	77	1,415
2	22,450	1,472	–3,176	–0.0097	0.0150	0.0193	3,612	2,023	–1,771
3	13,863	–294	18,577	0.0607	0.0091	–0.0454	2,916	1,390	653
4	11,472	1,748	6,151	0.0204	0.0110	0.0032	1,835	482	1,005
5	16,926	1,316	–2,412	0.0065	0.0061	0.0178	2,638	812	–183
6	10,343	5,883	1,517	0.0121	0.0215	0.0081	751	616	1,369
7	9,183	–602	11,263	0.0084	0.0061	0.0128	1,421	–91	1,270
8	19,413	6,211	–4,332	0.0088	0.0069	0.0091	2,886	671	–834
9	19,408	4,437	1,623	0.0049	0.0122	0.0096	2,796	587	–187
10	19,960	4,609	2,400	0.0295	0.0112	–0.0283	2,831	592	–114
11	27,067	2,565	–5,996	0.0234	0.0070	–0.0257	3,841	261	–396
12	21,246	9,070	4,104	–0.0046	0.0099	0.0142	3,002	1,130	215
13	19,391	–879	–737	0.0255	0.0139	–0.0277	2,491	–44	–75
14	26,989	1,814	–2,864	0.0175	0.0102	0.0026	4,016	508	–1,098
15	27,155	5,182	–11,584	0.0172	0.0145	–0.0014	4,637	634	–2,309
16	19,682	8,030	–2,069	0.0181	0.0165	–0.0068	3,686	1,599	–1,623
17	20,617	2,170	142	0.0138	0.0115	0.0038	1,883	172	1,286
18	17,375	–186	–9,501	0.0315	0.0099	–0.0189	3,019	–190	–1,455
19	21,626	4,220	–180	0.0116	0.0096	–0.0091	2,922	263	–772
20	23,284	3,093	913	0.0163	0.0051	0.0022	2,931	333	547
Pooled	17,966	3,522	1,238	0.0156	0.0121	–0.0019	2,546	533	96

[a] Components for milk expressed as hundreds (last two digits rounded).

and minus values consistent with sampling about a mean of near zero. In contrast, the sire and herd components present few negative estimates suggestive of sampling about a positive value of reasonable magnitude when it is realized that the herd components are based on substantially more information.

When the herd, sire, and residual components for fat per cent and milk in Table 2 are considered, breed differences in the components are evident. The components for fat per cent in Holsteins are each approximately one-half the magnitude of the corresponding variance components for Guernseys and Jerseys. Although the differences are not so striking for milk, the components for Holsteins are larger than the corresponding components for the two other breeds. For fat yield, where the means for the three breeds are of the same order of magnitude, the components are much more similar.

The variances presented in Table 2 are of interest to aid in planning progeny testing programs such as have been considered by Dickerson and Hazel *(2)* and Robertson and Rendel *(14)*. One requisite to such planning with progeny testing is an evaluation of the regression of future daughters on the performance of the first tested daughters. Where the performance of only one daughter is used to predict the average performance of many future daughters each in different herds, the regression coefficient can be written:

$$\frac{\sigma^2_s}{\sigma^2_s + \sigma^2_h + \sigma^2_{hs} + \sigma^2}$$

where σ^2_s, σ^2_h, σ^2_{hs}, and σ^2 refer to the sire, herd, herd by sire, and residual variances, respectively. The expected regression for specific situations can be evaluated if reliable estimates of the several variances are available. As an example, where there are n first daughters each in a different herd, the regression of future daughters in different herds on the first tested daughters can be written:

$$\frac{\sigma^2_s}{\sigma^2_s + \dfrac{\sigma^2_h + \sigma^2_{hs} + \sigma^2}{n}} = \frac{n}{n + \dfrac{\sigma^2 + \sigma^2_{hs} + \sigma^2}{\sigma^2_s}}$$

Substituting the values for the components given in Table 2 gives the regression coefficients presented in Table 5. These regression coefficients based on intra-state variances indicate the portion of the superiority or inferiority of n unselected daughters of a bull that would be expected to be manifest in a large

TABLE 5

Values for the regression of a large number of future daughters on the performance of n *first daughters each tested in different herds*

Breed	Milk	Per cent	Fat
Guernsey	$\frac{n}{n+14.0}$	$\frac{n}{n+6.3}$	$\frac{n}{n+17.4}$
Holstein	$\frac{n}{n+16.0}$	$\frac{n}{n+7.4}$	$\frac{n}{n+13.8}$
Jersey	$\frac{n}{n+12.1}$	$\frac{n}{n+7.5}$	$\frac{n}{n+14.9}$

number of future daughters of the sire. Despite the divergence in magnitude of the variance components for the three breeds, the regressions show striking similarity. Values for milk and fat are comparable, but the regressions for fat per cent are higher, indicating more genetically conditioned variance in this trait.

DISCUSSION

Even though there was little indication of genotype-environmental interaction for the production traits studied, this should not be interpreted to mean that such interactions do not exist. Much more precision in defining both the genotypes and the environments would be required, however, before interpretation of such interaction effects could be made. The classification of environments in terms of herds of necessity lacks precision; yet this is the existing situation. The herd might be characterized by the peculiarities of the herdsman, feed, milking, and management practices, which are measured in composite and do not present the distinctness that might be obtained in an experiment designed to test a specific interaction effect.

Bonnier *et al. (1)* and Hancock *(8)* have reported experiments with identical twins to test more specifically one environmental condition, namely, level of energy intake. In the experiments of Bonnier *et al.* twins were permitted low

and high energy intakes. Other twins were managed with no treatment differences, thereby allowing an estimate of error uncomplicated by twin-treatment interaction. Interaction effects were present for milk yield during first lactations, but these effects diminished during the second and third lactations. Hancock's experiment indicated the combined interaction and error variance for casein and fat per cent, milk, casein, and fat yield to be small. Intra-season rank correlations between twins on different levels of energy intake were also high. Since these workers used identical twins, the whole of the genotype-environmental interaction effect should have been expressed rather than only the portion associated with transmissible genetic influences common to paternal sisters.

Gowe and Wakely *(4)* studied possible strain-location interactions in poultry with some concern as to whether or not such interactions were of sufficient magnitude to question seriously the validity of centralized random sample egg production tests. Their locations in many respects would correspond to the herd designation for cattle. These authors did not find significant strain-location interactions for several measures of egg production. Work by Gutteridge and O'Neil *(5)* was in agreement with these results on production traits. Gowe and Wakely, however, did find strain-location interaction for mortality. Osborne *(13)* also found evidence for interaction of sire families and hatching dates as influencing days to sexual maturity in Brown Leghorns.

Since the sire by herd interaction variance for production traits in the dairy data studied was small, not only sire by herd effects but also possible nicking effects undoubtedly were of little import. This is in agreement with studies already cited *(11, 15)*. It should be pointed out, however, that the contribution from nicking would be realized in the present study only as the herds themselves presented genetic distinctness as a background for the sires' genotypes and might not be representative of nicking of specific sires and dams.

The conclusion that sire by herd interaction effects are not of major concern is strengthened somewhat since most of the possible confusing circumstances in these data would augment the estimate of sire by herd interaction variance. One such consideration is the possibility that the choice of the dams to which sires were mated in a given herd was not entirely a matter of chance. The departure from randomness in this respect would be restricted by limitations in the choice of available semen; hence, during the period covered by this study special matings would not be anticipated to have an important influence on increasing the interaction variance.

Although the data were restricted to a 5-year period with over 70% of the records being made during 2 years, some contribution of yearly influences toward increasing the sire by herd interaction variance also might be anticipated. This contribution would result from intra-herd yearly changes where all the sires in the herds were not used contemporaneously, rather than from a general time trend.

Even though the evidence from this study does not suggest large sire by herd interaction effects, the influence of interactions of the magnitude of those indicated for milk and fat in Holsteins would warrant attention to increasing the

number of herds represented in the proof of sire to be used subsequently in a different herd or herds. Such interactions represent another reason for overestimates of additively genetic variances, especially where half-sib correlations on an intra-herd basis are employed; thus, estimates of heritability in the narrow sense could be influenced substantially. It is of interest to note that the variances among the sires in this study, which were chosen for use in artificial insemination, are comparable to variances found among bulls used in natural service.

With the restriction of the analysis to an intra-state basis, consideration could not be given to the broader regional aspects of hereditary-environmental interactions. Only a meager amount of data bearing on this point was available. It is hoped that as additional data become available, further inquiry into this point may be permitted among the progenies of sires used in the North Central dairy areas and also in the South.

SUMMARY

Lactation production records of 24,754 daughters of Guernsey, Holstein, and Jersey sires used in artificial breeding associations throughout the United States were studied to assess the magnitude of the sire by herd interaction variances for milk, fat per cent, and fat yield. Only records initiated during the postwar period from 1946 to 1950 were included, and records contributing to a sire's proof prior to entering artificial service were not used.

Values of variance components for the individual breeds indicated sire by herd interaction variances for test to be inconsequential, with the possible exception of the Jerseys, where the sire by herd interaction variance approached the magnitude of the sire variance. The more limited data for Jerseys, however, afforded an opportunity for a larger sampling error than in the other breeds. Sire by herd interaction variances for milk and fat were indicated as zero or nearly so, and positive estimates were obtained only for the Holsteins. These variances for Holsteins were approximately one-third and one-fifth the magnitude of the corresponding sire components for milk and fat, respectively.

Limitations of the data are discussed, but, inasmuch as most of the confusing circumstances would tend to augment the sire by herd variance, it is apparent that specific sire by herd differences are not of major importance in these data. The ranking of a group of bulls, based on their daughters' performance in a given herd, would be expected to be substantially the same when these same bulls are compared on the basis of daughter performance in other herds in the same state.

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EFFECT OF FEEDING VARIOUS LEVELS OF N,N′-DIPHENYL-PARA-PHENYLENEDIAMINE TO LACTATING DAIRY COWS AND ITS DETECTION IN THE MILK[1]

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It has been previously reported *(6)* that the feeding of 0.01% of N,N′-diphenyl-para-phenylenediamine (DPPD) in the ration prevented the incidence of copper-induced oxidized milk flavor and appeared to increase carotene utilization. The present study was undertaken to ascertain the effect of various levels of DPPD on the aforementioned criteria as well as the possible mammary transfer of DPPD.

EXPERIMENTAL PROCEDURE

Twenty cows, five Guernseys, and 15 Holsteins, were selected for producing milk that exhibited oxidized flavor 72 hours after the addition of 5.0 p.p.m. Cu^{++}. After a 1-week standardizing period, the cows, in groups of five with restriction as to breed, were randomly assigned to one of five levels of DPPD (feed-grade) supplementation during a 5-week comparison period. The levels were 0.0000, 0.0001, 0.0010, 0.0100, and 0.1000% DPPD of total ration fed (90% dry matter basis), resulting, respectively, in average daily intakes of 0.000, 0.019, 0.184, 1.839, and 20.203 g. of DPPD. The DPPD was premixed with linseed oil meal and included in the grain allowance at each evening feeding. The animals were fed on the average 38 lb. grass silage, 14 lb. alfalfa hay, 12 lb. dairy ration, 3 lb. citrus pulp, and 0.1 lb. linseed oil meal.

At weekly intervals during the standardizing and comparison periods, samples of a morning and an evening milking were composited for determination of specific gravity, butterfat content, and concentration of carotenoids and vitamin A. Blood samples were obtained weekly for determination of carotenoid and vitamin A content. Twice each week during the standardizing and comparison periods milk samples were taken for organoleptic testing without and with 1.0 and 5.0 p.p.m. Cu^{++} as copper sulfate. These samples were tested independently by two judges for the presence or absence of oxidized flavor 72 hours after milking. Analytical and statistical procedures for the above criteria were similar to those previously reported *(6)*. One Guernsey fed 0.0001% DPPD had to be removed from the experiment because of sickness, and the missing values were calculated.

DPPD in the milk was determined qualitatively *(2, 3)* by saponifying the weekly 20-ml. milk samples with 30 ml. of alcoholic KOH for 3 hours at room

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temperature. The saponification mixtures were pooled on an individual cow basis, and the resulting mixture, representing 100 ml. of milk, was diluted with an equal volume of H_2O. After addition of 3 to 4 g. Na_2SO_4, extraction with 50 ml. benzene by vigorous shaking for 5 minutes and subsequent centrifugation, 5- to 30-ml. benzene aliquots were evaporated under N_2 on a steam bath to a volume of 3 to 5 ml. and then to dryness at room temperature. The residue was taken up in 10 ml. of acetone, and four drops of concentrated HNO_3 were added. Red color intensity was observed visually after 3 minutes, and each sample was assigned an intensity value as follows: 0 for no red color, and 1, 2, and 3 for increasing intensities. Confirmatory tests made with $CuSO_4$, which results in a blue color in the presence of DPPD *(5)*, were run on milk samples from cows fed the highest level of DPPD.

RESULTS AND DISCUSSION

Actual pounds of milk and specific gravity (Table 1) were unaffected by level of DPPD in the ration, whereas the fat percentage was greater in all groups receiving DPPD than in the controls ($P < 0.05$). Although the pounds of fat and FCM reflected in general the increased fat percentage due to DPPD, these criteria were found not to be statistically significant. Similar increases in fat percentage were observed in one of two experiments in the previous report *(6)*

TABLE 1
Effect of level of DPPD on milk production

	Per cent of DPPD in the ration[a]					Standard deviation of a cow
	0.0000	0.0001	0.0010	0.0100	0.1000	
Lb. of milk						
Standardizing[b]	57.4	35.2	31.8	32.5	48.7	15.2
Comparison[c]	53.3	32.3	29.3	29.1	45.0	14.9
Adjusted comparison[d]	37.5	38.0	38.4	37.6	37.6	2.3
% fat						
Standardizing	3.9	4.0	4.3	4.0	3.6	0.4
Comparison	3.8	4.1	4.2	4.0	3.8	0.4
Adjusted comparison	3.8	4.1	3.9	4.0	4.2	0.1
Lb. of fat						
Standardizing	2.199	1.406	1.324	1.282	1.678	0.548
Comparison	1.983	1.361	1.205	1.160	1.679	0.525
Adjusted comparison	1.401	1.522	1.443	1.437	1.585	0.125
Lb. of FCM						
Standardizing	55.92	35.20	32.55	32.20	44.62	4.48
Comparison	51.08	33.36	29.79	29.05	43.17	13.76
Adjusted comparison	35.93	38.05	37.02	36.62	38.84	2.67
Specific gravity						
Standardizing	1.032	1.034	1.033	1.033	1.032	0.010
Comparison	1.031	1.033	1.033	1.032	1.032	0.010
Adjusted comparison	1.032	1.032	1.033	1.032	1.032	0.001

[a] 90% dry matter basis.
[b] Average of a 1-week period prior to the inclusion of DPPD in the ration.
[c] Average of a 5-week period during which DPPD was included in the ration.
[d] Values of the comparison period adjusted for those of the standardizing period.

and may possibly be attributed to changes in lipid metabolism. Bunnell *et al.* *(4)* reported higher liver lipid concentrations in DPPD-supplemented chicks, and this also suggests an alteration of lipid metabolism.

Both plasma carotenoid and plasma vitamin A concentrations tended to be greater in the groups fed DPPD than in the control; however, these differences did not approach statistical significance (Table 2). Inappreciable differences were observed for milk carotenoids. Concentration of vitamin A per 100 ml. of milk or per gram of fat and the total daily secretion of vitamin A were considerably increased by the addition of DPPD to the ration; this was especially pronounced at the higher levels of DPPD.

TABLE 2

Effect of level of DPPD on apparent carotene utilization

	Per cent of DPPD in the ration[a]					Standard deviation of a cow
	0.0000	0.0001	0.0010	0.0100	0.1000	
Plasma carotenoids			*(γ/100 ml. plasma)*			
Standardizing[b]	878	834	837	822	928	226
Comparison[c]	795	722	755	707	951	205
Adjusted comparison[d]	761	753	761	752	789	54
Plasma vitamin A						
Standardizing	26.8	33.7	27.8	29.4	31.2	7.4
Comparison	24.2	30.8	26.8	25.9	30.3	5.5
Adjusted comparison	26.2	28.1	28.2	26.2	31.2	2.4
Milk carotenoids						
Concentration			*(γ/100 ml. milk)*			
Standardizing	17	18	15	15	23	8
Comparison	18	20	16	15	24	7
Adjusted comparison	19	19	18	17	20	5
Per gram fat			*(γ/g fat)*			
Standardizing	4.1	3.5	3.4	3.7	5.9	2.1
Comparison	4.5	3.7	3.7	3.6	5.7	5.5
Adjusted comparison	4.5	4.1	4.2	3.8	4.6	1.3
Total daily secretion			*(γ)*			
Standardizing	3480	2743	2133	2123	4596	1651
Comparison	3627	2791	2119	1892	4045	1212
Adjusted comparison	3378	2936	2592	2370	3198	930
Milk vitamin A						
Concentration			*(γ/100 ml. milk)*			
Standardizing	10.3	10.0	10.0	10.3	9.7	2.6
Comparison	11.9	13.7	13.6	14.5	26.2	2.5
Per gram fat			*(γ/g fat)*			
Standardizing	2.58	2.55	2.28	2.55	2.75	0.69
Comparison	3.08	3.28	3.22	3.54	6.82	0.82
Total daily secretion			*(γ)*			
Standardizing	2532	1545	1370	1388	2045	507
Comparison	2760	1872	1773	1836	5300	1405
Adjusted comparison	1524	2208	2388	2422	4839	1405

[a] 90% dry matter basis.

[b] Average of a 1-week period prior to the inclusion of DPPD in the ration.

[c] Average of a 5-week period during which DPPD was included in the ration.

[d] Values of the comparison period adjusted for those of the standardizing period.

TABLE 3

Effect of level of DPPD on incidence of copper-induced oxidized milk flavor and detection of DPPD in the milk

		Per cent of DPPD in the ration[a]					Standard deviation of a cow
		0.0000	0.0001	0.0010	0.0100	0.1000	
Raw milk		*(% of samples free from oxidized flavor)*					
Standardizing[b]		75	100	100	100	100	23.35
Comparison[c]		94	100	99	100	99	5.90
Raw milk + 1.0 p.p.m. Cu^{++}							
Standardizing		11	18	25	0	25	34
Comparison		24	92	92	98	98	14
Raw milk + 5.0 p.p.m. Cu^{++}							
Standardizing		0	0	0	0	0	0
Comparison		4	8	11	95	94	12
DPPD in the milk							
Intensity of red color[d]	Group I	0	–	1	2	3	–
	II	0	0	1	2	3	–
	III	0	0	0	2	3	–
	IV	0	0	1	2	3	–
	Mean	0	0	0.8	2.0	3.0	–
		$\sqrt{\text{Intensity of red color} + \frac{1}{2}}$					
	Mean	0.72	0.73	1.10	1.58	1.87	0.12

[a] 90% dry matter basis.

[b] Average of a 1-week period prior to the inclusion of DPPD in the ration.

[c] Average of a 5-week period during which DPPD was included in the ration.

[d] Intensity of red color due to nitric acid test *(2)* observed visually.

The incidence of oxidized milk flavor, expressed as percentages of samples free of the flavor defect, is given in Table 3. All levels of DPPD resulted in lowered incidence of oxidized flavor when either 1.0 or 5.0 p.p.m. of Cu^{++} were added to the milk. As little as 0.0001% of DPPD was effective when 1.0 p.p.m. of Cu^{++} was present and 0.0100% when 5.0 p.p.m. of Cu^{++} were present.

DPPD was found qualitatively (Table 3) in all samples of milk from cows fed 0.0100 and 0.1000% DPPD in the ration and in three out of four samples from cows fed the 0.0010% level of DPPD. Quantitative estimation of DPPD was possible only in the samples from cows fed the highest level of DPPD, since under the experimental conditions used above, when aliquots of the benzene extract exceeding 5 ml. in volume were evaporated, cloudiness developed on dissolving the residue in acetone and subsequent treatment with nitric acid. In samples from cows fed the highest level of DPPD, absorbancy (2-log G) was read on an Evelyn photoelectric colorimeter equipped with a 490-mμ filter, and concentrations were determined by reference to a standard curve obtained from solutions of purified DPPD (B. F. Goodrich Chemical Co.). Estimates of DPPD in the milk were 30, 30, 40, and 30 γ/100 ml. milk, respectively, for the four cows, which represented on the basis of these analyses a mammary transfer of approximately 0.032% of the DPPD fed. The presence of DPPD in the milk from the cows fed the highest level was confirmed by the blue color test made with $CuSO_4$ *(5)*.

In consequence of the detection of DPPD in milk of cows receiving various dietary intakes of this antioxidant and the reports that DPPD interferes under certain analytical conditions with the spectrophotometric estimation of carotene *(1, 5)* and with the colorimetric estimation of vitamin A by the Carr-Price reaction *(5)*, it was necessary to estimate the magnitude of this interference. For this purpose standard solutions containing in a total volume of 11 ml. of petroleum ether (b.p. 30°-60°) and benzene (10 : 1): 0, 2.2, and 9.6 γ of β-carotene (General Biochemicals), 0, 1.0, and 5.8 γ of vitamin A alcohol (General Biochemicals) and 0, 6, and 37 γ of purified DPPD (B. F. Goodrich Chemical Co.) were read in all combinations at 440 mμ for β-carotene and, after evaporation in vacuo, at 620 mμ for vitamin A by the Carr-Price reaction. Based on two replications, absorbancy (y_1) at 440 mμ was best expressed by the following equation: $y_1 = 0.00054 + 0.03449\,x_1 + 0.00002\,x_3 \pm 0.00134$ where $x_1 = \gamma$ carotene in the sample and $x_3 = \gamma$ DPPD in the sample and vitamin A (x_2) not contributing significantly at this wave length. Similarly, absorbancy (y_2) at 620 mμ for the Carr-Price reaction was $y_2 = 0.00068 + 0.00331\,x_1 + 0.07066\,x_2 + 0.00028\,x_3 \pm 0.00305$. Feed grade (95%) DPPD gave essentially similar functions. These results confirm previous work *(5)* in regard to the interference of DPPD in the estimation of vitamin A by the Carr-Price reaction and in addition indicated that DPPD may contribute to absorbancy at 440 mμ. However, the magnitude of the increases for carotenoids in the plasma and for vitamin A in the plasma and milk was larger than could be accounted for by the above relationships. Methods of estimating or eliminating DPPD, as well as the possible effects of the analytical procedures used in carotenoid and vitamin A analyses on the chromogenic activity of DPPD, need to be investigated more adequately before indisputable evidence as to the effect of DPPD on carotene utilization can be obtained.

SUMMARY

Twenty cows, five Guernseys and 15 Holsteins, were selected for producing milk which exhibited oxidized flavor 72 hours after the addition of 5 p.p.m. Cu^{++}. After a 1-week standardizing period, the cows were fed one of five levels of DPPD, 0.0000, 0.0001, 0.0010, 0.0100, or 0.1000% of the total ration fed (90% dry matter basis) for a 5-week comparison period. All levels of DPPD resulted in significant increases in per cent fat of the milk. Similarly, milk vitamin A was significantly increased. All levels of DPPD reduced the incidence of oxidized flavor in the presence of 1 p.p.m. of Cu^{++} and the two highest levels in the presence of 5 p.p.m. of Cu^{++}. DPPD was qualitatively found in milk of the cows fed the three highest levels of DPPD and quantitatively in the highest level. In the last case, the concentrations of DPPD averaged 32.5 γ/100 ml. of milk, which represented a mammary transfer of 0.032% of the DPPD fed.

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TECHNICAL NOTES

TRIBUTYRIN LARD AND TALLOW IN FEEDING OF "FILLED MILK" FOR VEAL PRODUCTION

Since Wiese *et al. (6)* obtained satisfactory daily gains by feeding "synthetic" milk diets ad libitum without hay or grain to veal calves, several investigations have been made in attempts to find improvements in this method of veal production. Gardner *et al. (1)* found that they could secure excellent daily gains with veal calves when only whole milk was fed ad libitum.

Wiese *et al. (6)* observed that animals which received rations prepared with lard as the source of fat showed good weight gains, did not scour, and appeared normal and healthy. Krauss *et al. (5)* reported an average total gain of 44 lb. when calves were fed a milk fat substitute–skimmilk combination along with grain and hay to 50 days of age as compared to 56 lb. gain for calves fed whole milk in the same period. Gullickson *et al. (2)* have shown that vegetable oils are not satisfactory substitutes for butterfat, and Jacobson *et al. (4)* showed that these could be greatly improved by hydrogenation. Since Hopper *et al. (3)* found that the gains obtained on a ration that contained butyrated lard were equal to those obtained on whole milk, it appears that the short chain fatty acids are important for the calf and that a butyrated lard "filled milk" can replace whole milk in feeding of young dairy calves.

The purpose of this experiment was to compare the gains of dairy calves on ad libitum feeding of whole milk with gains made on "filled milk" rations containing tributyrin lard and tallow as the sources of fat.

Tallow[1] and tributyrin lard[2] were utilized as sources of fats in "filled milk" rations which were compared to whole milk in this trial. These fats were emulsified[3] and added to reconstituted skimmilk to make a 4.0% fat milk, which was warmed before feeding. Thirty bull calves were removed from their dams at 3 days of age and placed in individual stalls bedded with straw. The calves were fed ad libitum from nipple pails three times daily. Any milk refused was weighed back after each feeding. The calves were removed from the test at 56 days of age.

Trace mineralized salt was provided at all times and a mineral solution that supplied 206 mg. ferric citrate, 10.5 mg. of $MnSO_4$, and 2.34 mg. of $CuSO_4$ per liter of milk was added at each feeding. Each calf received by capsule 100,000 I.U. of vitamin A and 100 I.U. of vitamin E weekly and 50,000 I.U. of vitamin D at the beginning of the trial and again at 4 weeks of age. The calves received 2 mg. of aureomycin hydrochloride per pound of milk.

The calves were weighed during the first 3 days of life and during the last 3 days of the experiment and weekly throughout the test. Calves of Holstein, Ayrshire, and Brown Swiss breeds and crossbreds were used. The animals were placed in the groups in such a manner as to have an even distribution of breeds among groups.

The growth data (Table 1) showed that ad libitum feeding of emulsified "filled milk" rations containing tributyrin lard and tallow gave average daily gains of 1.69 and 1.61 lb., respectively, as compared to 1.80 lb. daily gains on whole milk over a 56-day period. Analysis of variance did not reveal any significant differences among the groups. These data show then that the calf can utilize tributyrin-lard and tallow as sources of fat to make gains essentially equivalent to those made on whole milk. The results point out that not only lard containing butyrate but also beef tallow can satisfactorily replace milk fat in the ration of the veal calf.

[1] The tallow utilized was that from beef carcasses.

[2] Tributyrin lard was lard to which tributyrin had been added at the level of 3½%.

[3] Tallow and lard emulsions were prepared by homogenizing in water with an emulsifier (lipogel) to give a 60% fat emulsion.

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TABLE 1

Growth of calves on whole milk and "filled milk"

Ration	No. of calves	Av. initial weight	Av. final weight	Total gain	Av. daily gain
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
Whole milk	10	95.80	196.68	100.88 ± 20.64	1.80
Tributyrin lard[a]	10	94.15	188.88	94.73 ± 12.93	1.69
Tallow	10	100.85	191.09	89.04 ± 15.27	1.61

[a] Lard with 3.5% tributyrin added.

due to Arthur Miller, Roy Shirley, and Richard Knight for feeding and caring for the calves. The aureomycin hydrochloride was supplied by the American Cyanamid Company through the courtesy of E. L. R. Stokstad.

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HEREDITARY EDEMA IN AYRSHIRES

A brief paper was published on "Hereditary Edema in Ayrshire Cattle" in the April, 1955, Journal of Dairy Science (**38**: 440-441). This young Ayrshire bull evidently was homozygous for hereditary edema. A large cyst was present in the pituitary gland with deficiencies in both the anterior and posterior lobes of the gland.

Since the publication of this paper, a second Ayrshire animal has been examined that was almost identical in age and appearance with the first one except that this animal was a female. In this heifer the pituitary gland also possessed a large cyst not significantly different from that described and pictured for the bull. Except for the edema no other abnormalities were observed.

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POTENTIAL SERVICES OF A BULL FREQUENTLY EJACULATED FOR FOUR CONSECUTIVE YEARS

The literature contains a number of reports on the effects of frequency of semen collection on sperm production and libido of dairy bulls, but no report has established a maximum frequency. Where fertility data have been obtained in connection with such studies, no critical change in fertility level has been attributed to the frequency of collection. Although it has been shown that sperm numbers in the bull are restored to normal 24 hours after exhaustive semen collections *(3, 4)*, it is still common practice in artificial insemination units to collect semen from each bull only once each week or even less frequently. Satisfactory semen production and libido are maintained in most bulls with weekly collections. Few attempts have been made to collect semen more frequently, although the demand for semen from valuable sires often would warrant such a practice if it were not detrimental to the bull and his fertility.

Data were reported previously from this laboratory on the effect of semen collection frequencies of once, twice, and thrice per week with young Holstein bulls *(1)*. All bulls on the two lower frequencies completed a year on this study without apparent reduction in libido or semen production. One bull on the thrice per week collections not only completed the first year of the study but has been continued at this semen collection frequency for four consecutive years. The data collected on this bull suggest that some bulls may have a greater capacity to produce sperm than has been recognized previously.

This Holstein bull first expressed sexual interest at the age of 25 weeks and first ejaculated at 38 weeks of age. For four consecutive years after the first ejaculation the bull was kept on a semen collection schedule of three times per week. Single ejaculates were collected on each Monday, Wednesday, and Friday. A false mount was allowed prior to the collection of semen to stimulate sexual excitement. Failure to mount within 10 minutes after approaching the teaser cow was considered as a refusal.

A summary of the data obtained during the 4-year period on this bull is presented in Table 1. It is believed that the increase in the number of refusals during the third and fourth years

Table 1

Semen data on a Holstein bull ejaculated three times per week for 4 years

Year	1st	2nd	3rd	4th	All
No. of ejacs.	153	151	143	135	582
Av. vol/ejac. *(ml.)*	2.8	3.8	5.0	6.0	4.3
Motile sperm *(%)*	56	59	62	62	60
No. sperm/ml. *($\times 10^6$)*	1,089	1,530	1,513	1,451	1,383
Total sperm produced *($\times 10^9$)*	523	898	1,093	1,197	3,711
Potential services[a]	52,289	89,781	109,312	119,676	371,058

[a] Based on inseminating 10×10^6 spermatozoa per insemination.

can be partially explained on the basis of temporary changes in collectors and barn personnel. With the return of the regular personnel, he resumed the usual frequency and showed no indication of a reduction in libido.

All ejaculates have been extremely uniform in seminal characteristics. Few samples have been obtained that would not be acceptable for shipment by most artificial insemination units. The number of abnormal spermatozoa has been exceedingly low. The presence of spermatozoa showing protoplasmic droplets has been negligible and there has been no evidence of an increase in the number of sperm showing this characteristic over the 4-year period.

At the age of 4 years and 9 months, the calculated number of spermatozoa this bull had ejaculated was 3711×10^9. Assuming the use of 10×10^6 spermatozoa for each insemination *(2)*, enough spermatozoa to breed 371,058 cows had been produced. Although the bull has not been used extensively for breeding purposes, he was proven fertile during his third year of semen production.

At the time of this writing, the bull is 5 years old and weighs 2,550 lb. He is considered to be in excellent breeding condition. He has been fed a 14% protein ration consisting of corn, oats, wheat bran, and soybean oil meal with hay that was relatively poor in quality. Pasture has never been supplied and a negligible amount of corn silage has been fed. Exercise was restricted to what the bull could obtain in an 8 by 12 ft. box stall and by going to the collection stall, a distance of 20 ft.

Although the data obtained with this one bull suggest that more intensive semen collection frequencies may be possible with some bulls, care and caution should be used in attempting to put other bulls on such a schedule. Frequently the lack of proper training, a prior inhibiting experience, or an inherent lack of sex drive may cause a bull to refuse to work at frequent intervals.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

After College—What?

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In the recently completed survey of the dairy manufacturing graduate situation in the dairy industry by H. B. Henderson of the University of Georgia, the need for industry training programs was stressed more than any other single item. Only about 46 out of 284 graduates reported that they were included in a program they would rate as "good." The main criticisms of the programs in use were a lack of organization, insufficient information on company policy, incomplete records of the trainee's progress, and a lack of reports required of trainees.

Other industries have taken the lead in developing training programs for the college graduates in their organizations. An American Management Association study, completed in 1954, showed that in some industries from 35 to 50% of the companies had extensive training programs. The United States Steel Corp., Armstrong Cork Co., General Motors, Sears-Roebuck, General Electric, Westinghouse Electric, Eastman Kodak, and many others have training programs that last from 9 months to several years and may cost the companies as much as $10,000 a year for each trainee. These organizations believe that developing good management is as important as developing a good product. Although a few companies apparently look for business "geniuses" to direct their activities, many more are concerned about the desirability of a strong, well coordinated management team. To this end the development of good management has become a major activity in these organizations.

Many college placement directors are recommending that their most capable graduates go with organizations having training programs, for it is their belief that such a program is the best insurance for developing a young man's full potential. Graduates are anxious to become associated with companies having training programs because they know they are less likely to become lost in such an organization. They learn while doing and are less likely to experience the "after-college slump," which so often occurs when there is no organized training program.

A superior training program can be an important factor in attracting and keeping the most capable college graduates. As has been said, "A company is known by the men it keeps." The better a man is, the more he recognizes the value of training; it is the weak man who is most likely to resist the idea. A company training program supplies two types of knowledge: *(a)* that which is gained by actual performance of work and *(b)* that which comes from the discussion of ideas and experiences. This combination of knowledge sources enables an individual to develop rapidly.

H. H. Judson of the Consolidated Edison Co. cited the following objectives of a worth-while executive development program:

1. To improve the competitive effectiveness of the company by:
   a. Assisting executives to function more effectively in their present jobs.
   b. Developing "generalists"—men who think from the over-all company standpoint rather than from a specialist's point of view.
   c. Making better use of executive abilities.
   d. Improving teamwork and morale in the management group.
2. To increase the effective productive life of executive personnel through accelerated development.
3. To build a reserve of qualified personnel to back up key positions.

Many persons consider training programs practicable only for large companies. This is not true, however, for small companies can do practically the same thing on a smaller scale. Small companies need executive development programs as much as, if not more than, the large ones. The small company is frequently left without a "boss" and with no one trained to take over. It is also frequently true that an executive in a small company has broader direct responsibilities than his counterpart in a large organization.

Training Programs

Training programs may be of two types: (1) training for lower levels of management and (2) training for junior and senior executives.

Trainees for lower levels of management are frenquently college graduates just entering the organization, but they may be employees al-

ready with the company who wish to advance to more responsible positions. The training period for this group may last as long as 3 years, depending on the size of the organization.

Trainees for junior and senior executives are usually selected from among employees who have superior work records and have demonstrated adequate growth potential. The individual's work record may be appraised by periodic merit ratings or performance reviews. His growth potential may be appraised by tests and interviews. Other factors frequently considered are age, position, and training background. Final selection is usually made by a committee of top management.

It is apparent that no two companies have exactly the same training program; yet the fundamental parts of all are similar.

Training for lower levels of management. The trainees may be introduced to the organization by taking a basic course outlining the principal parts of the company operation. After this, the trainees may spend from several weeks to several months in the following departments: plant production, quality control, time study and methods, engineering, sales, and industrial relations. Industrial relations may include safety, wage and salary surveys, workmen's compensation, grievances, and labor contracts. Actual line work and association with union stewards, shop foremen, and rank-and-file workers are usually considered very important. During this time the trainees in some company programs meet weekly with company advisors and may participate in other evening educational activities.

The trainees are frequently required to submit reports whenever a specific phase of the course is completed. Job breakdowns are considered to be important. Some companies have received very helpful suggestions as a result of this phase of the program. Trainees are usually evaluated periodically by their immediate superiors in each phase of the program. Management holds periodic performance reviews and counsels with trainees to help correct weak points.

Some companies have worked out training schedules so detailed they can tell exactly in what phase of training an individual will be on any date of the training program. Others vary the program a little more to fit the past experience and training of the individual.

Most companies do not depend on one training technique but use many approaches, such as the following:

Visits to other companies.
Attendance at technical meetings.
Management development conferences.
Reference reading on management topics.
Group meetings.
Committee assignments on management problems.
Understudy to supervisor positions.
Individual counseling.
Use of management consultants.
Community activities.
Health and fitness programs.

When a trainee completes this phase of training, he should be ready for some type of supervision where his performance will help determine if he has the qualifications that will make him eligible for further training.

Training for junior and senior executives. Multiple management provides for the participation of many persons in management in contrast to control by one man or a small group of men. A junior board of directors may be formed to study company problems and make recommendations to the senior board. It may study any company matter it wishes. The board is usually appointed by top management to start with, but after it is once formed, it is self-perpetuating. Being elected to the junior board is an honor most employees seek, since board membership is a "proving ground" for an individual to see what he can do. Reelection is good evidence that the employee can contribute ideas and work satisfactorily with others and is considered to be a man of promise.

Company business management training schools are frequently held for junior executives during the summer. Business school instructors are usually hired to teach the courses, though the instructional staff may be supplemented by company executives. A course such as this may last several weeks.

Professional management organizations offer training courses in development. The American Management Association offers such a training course, which is used by many companies.

University executive development courses offer another means of training that is becoming increasingly popular. One of the most extensive courses of this type is that offered by the Harvard Business School. This course is 16 months in duration and leads to the degree of Master of Business Administration.

The individual's training program. If no company training program is available, it may be necessary for the individual to work out one of his own. Many of the techniques used in company programs can also be used by the individual. A look at the areas covered by courses for training executives at leading universities will help shape a self-training program. An individual can cover the same areas through planned reading, night schools, or correspondence. The subjects generally fall into the following categories:

Personnel and labor relations.
Production management.
Administration practices.
Business and economics.
Cost and financial administration.
Market management.

Companies with active programs have found that certain precautions should be observed in establishing any training program. The more important of these are:

1. Everyone in the organization should know what is going on.
2. The program should be open to everyone.
3. Participation should not be regarded as a guarantee of advancement.
4. The entire training program should not be turned over to a group of outsiders.
5. The major part of a program should not be of an observational nature.
6. There should be more than one training procedure.
7. Not all trainees should be put through the same program.
8. The training programs should stem from top management.

The problem of morale among employees is critical. Wallace Jamie, personnel director of the Carnation Company, reports that in a few instances training programs have been abandoned because this problem became serious (*J. Dairy Sci.*, **37**: 116. 1954). It developed because a feeling was generated among employees that the trainee group was a chosen clique, membership in which was a prerequisite to success. Jamie lists four methods of combating this problem.

The day of the haphazardly trained manager is gone. A manager trained in such a fashion may have been good enough in the past but is not good enough for the future. Napoleon once said, "There are no bad regiments—only bad colonels." It might also be said, "There are no bad companies—only bad managers."

If the dairy industry is to have the leadership it needs to meet the challenges of the future, it must make full use of its college-trained men. Industry training programs offer an excellent means of attracting and keeping the most capable college graduates.

Developing and Maintaining Personnel Morale

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Few, if any, discussions among American executives or employees go very far before the terms "morale" or "human relations" are used or implied. These terms crop up with equal frequency in writings of management or labor-management relations. All this adds up to one thing—among those persons just mentioned these terms are as important as is weather in most general conversations. It is proposed, therefore, to discuss several aspects of employee morale, and human relations.

Definitions

Let us turn first to a definition of "morale." It has reference to a state of mind which determines the attitude of an individual or a group of individuals toward an organization or its leaders (at any or all levels). Such attitudes determine in turn the willingness to cooperate effectively in the attainment of the objectives of the business. And therein lies the importance of morale—it has a most significant effect upon the success (or failure) of a company. Thus, morale may be but a state of mind but it nonetheless has tremendous concrete results. Any executive who attempts to operate without due consideration to it or its development handicaps himself severely in the competitive race. Moreover, he burdens himself unduly with many executive duties that cooperation would otherwise make unnecessary.

"Human relations" merely recognizes, on the other hand, other broader and more specific aspects of morale. This term refers to all the practices which seek to gain the cooperation of individuals as they work together. Such cooperation may be influenced by emotional, logical, sociological, and psychological aspects as well as by economic or political factors. Such cooperation has reference between executives and subordinates as well as among individuals at the same horizontal plane in an organization structure. In a nutshell, human relations refers to "getting along with others" so that the business may get along more effectively in this competitive world.

Thus, in human relations the executive must seek both to reduce conflicts among people as people, and to increase personal cooperation among these people. These goals are sought as they relate to human aspects of working together, not to the technological or machine aspects of industry. And these goals are sought for a very simple but important reason—the success of a company depends in the highest degree upon their attainment.

Theory of Morale Development

To work satisfactorily toward the development of good morale, one must have an adequate explanation (that is, theory) of the way morale develops and manifests itself. Such an

explanation is essentially one of cause and effect—that is, what causes it and what are its effects?

As already noted, morale is a state of mind which sets off certain reactions. If time permitted, it would be useful to look at some examples of "good" morale and see why it is good. We would find that all these situations reveal a common factor—understanding! All members in a situation of good morale understand that for them to be successful the company must be successful. Specifically high output, high effort, and good profits will then make possible personal advancement, security, recognition, good working conditions, and high wages.

Understanding of the dependence of personal success upon company success is the foundation of good morale. As a consequence, employees with good morale are willing to subordinate themselves for the good of the company. But by means of such subordination they know they will assure themselves of their own personal advancement. They see the relation between their own interests and those of the company for which they work. And when success comes to the company, the employees share in that success.

If good morale is developed in this way, what of it? The rest of our theory explains the effects of good morale. These effects include a willingness to follow orders with enthusiasm, willingness to follow company orders and executives, and willingness to work hard in the face of difficulties and obstacles. There are also such excellent effects as pride in company, respect for executives, initiative, alertness, and self-satisfaction. And what good are these effects? Why, they are the causes in turn of such desirable results as high output at low cost with a minimum of executive overhead—all of which determine in the final analysis the excess (if any) of revenues over expenditures, in other words, profits.

Thus, we can summarize the theory of morale in a few short sentences. Morale is a state of mind of individuals or groups. It develops from the process of understanding the relation between company objectives and personal interests. From this there flow effects which raise the willingness to cooperate effectively. As a consequence, the company is in a better position to attain its goals as well as to help employees attain theirs. This is selfishness on the part of both, but note that it is intelligent and cooperative selfishness that helps both parties.

Factors of Morale Development

Specifically what brings about this good "state of mind" and this good "understanding?" Very simply, anything may affect a person's attitude. Thus, everything is important in morale development. Just to review "everything," a useful check-list would cover the following: (1) The objectives of a company or any of its divisions, (2) the ethics that govern a company's actions, (3) the kinds and characteristics of leadership it exercises, (4) how and what functions are performed by the company, (5) the kinds of people a company hires, and how they act, (6) the physical and social environment of the company operations, (7) the nature and goodness of its policies, (8) the kind of organization structure it has, (9) the kinds of procedures used in the company, and (10) the manner in which the management plans, organizes, and controls the activities of the company.

Each of the foregoing could be subdivided into many details. The point is obvious: There is nothing that an executive can afford to overlook as having a possible effect upon morale. The only question is how to isolate particular factors that may be significant in a particular company at a particular time.

Determination of Current Morale

To know what to do about morale, we must first know what it is in a given case. Morale is generally measured in either of two terms: actual results or attitude of subordinates. First we shall discuss what is measured and then we shall take up how they may be measured.

Measures of morale: A very common way of measuring morale is to keep records on such results as output, quality, costs, performance, and expenditures. These are rather indirect measures of morale because they are in part results of morale, not a direct measure of a person's state of mind. Another group of measures gets closer to morale by keeping records of grievances, complaints, disciplinary actions, and similar types of personal difficulties.

More direct measures of morale may be sought by seeking to find out what employees really are thinking about. By any one of a number of methods, information may be sought on what employees think about such things as: job security, possibilities for promotion, seniority versus merit for promotional purposes, wage and salary policies of the company, working conditions, recreational, social, and athletic programs, economic and insurance programs, health programs, hiring and training methods, relationships with superiors and staff departments, community facilities, and governmental and legislative aspects.

By gathering opinions, attitudes, and feelings about such subjects, it is possible to gain not only a useful measure of morale but also specific points of irritation and of relative satisfaction. For example, let us assume that employees voice dissatisfaction with a wage policy. Rightly or wrongly, that is how they feel about the subject. We have, therefore, pin-pointed a measure of morale. What we do or can do about the subject is another matter. At least we know specifically what is bothering the employees.

Means of measuring morale. There are three common ways of measuring morale. These are by observation, interviews, and questionnaires.

Observation of people is a simple method, but it can be very effective. By careful observation of behavior and mannerisms and of individual output and results, useful deductions can be made. It is surprising how much people tell us about themselves if we learn to be consciously alert for the messages.

A more formal method of morale measurement is that of interviewing. Under this method, executives or trained interviewers talk things over with employees. This method has been used with great interest in the United States and has served to give management a surprising number of ideas about the thinking and attitudes of their employees.

The questionnaire has also been used to measure morale. For a number of topics about which it is desired to learn attitudes, a number of alternative answers are prepared. For example, we may ask—"What do you think about the fairness of your immediate superior?" Then we provide alternative answers with space for checking, as follows:

He is always fair ___
He is more fair than unfair ___
He is more unfair than fair ___
He is invariably unfair ___

The results can be tabulated statistically. Of course, the opinions of individuals must not be identified or the answers will not reflect what the people really think.

Plans of Improving Morale

Having determined what the status of morale is, it is then possible to attack the problem of what, if anything, should be done to improve it. What can be done usually depends upon what is wrong. Normally, low morale stems from three major sources: (a) some policy or program of the company or an executive is wrong, (b) an individual is misinformed about some policy or practice, or (c) an individual holds opinions contrary to those held by his company.

Each type requires a different treatment. When the company or executive is wrong, the course is clear: Correct this mistake as soon as possible. When an individual is misinformed, a program of education is called for. But when an individual disagrees with the company, the course is not so easy. Either try to reeducate the worker or, if possible, remove him from the payroll. All of this is easy to say, but it is really here that the work of morale development is never-ending. One cannot sit back and say—now I have good morale. Good morale can be developed only after hard work, and it can be lost overnight. Hence, eternal vigilance is the price of good morale, but a price that every executive should be willing to pay.

Handling Human Relations

Let us see what can be done more specifically to turn good morale into good human relations. What can we do to get along better with others? What can we do to smooth the points at which people meet, to reduce resulting frictions and to minimize areas of conflict?

So much has been said and written about these subjects that it would take weeks to discuss them adequately. Fortunately, our suggestions can be grouped into three major categories so that while not all problems of human relations can be discussed here, the major categories can. Thus, it has been found that human relations has three major aspects: An individual is dissatisfied with the company (a grievance); the company is dissatisfied with the individual (a disciplinary action); or both are relatively satisfied with each other, and the management wants to maintain this relationship (positive motivation).

Meanings and implications. Starting first with grievances, it is wise to define the term. First, we know that a grievance is an employee dissatisfaction. Second, however, its source or nature may vary considerably. For example, a grievance may be: expressed or unexpressed, written or oral, fair or unfair, valid or invalid, logical or just felt or believed, and company-induced or induced by out-of-company situations.

Each company must determine what limits it will place on areas of irritation which it will handle. Some people limit them to written statements pertaining strictly to company business. Others say "the sky is the limit." A restrictive policy merely drives conflicts underground. Sooner or later they suffer from the loss of effectiveness that results because employee dissatisfactions have not been cleared up. However, those who unduly open the gates for complaints, only ask for trouble. Usually a definition must be chosen that is in line with company ability to handle grievances, and their importance.

Disciplinary action refers to areas of conflict in which an executive is dissatisfied with an individual to the point that he deems corrective action necessary. This implies that an individual is not willing to obey orders or does not cooperate as effectively as he should and can. Corrective action is concerned with force, compulsion, and penalties. Hence some might argue that it is negative leadership. When over-used, the charge is correct. Yet it cannot be denied that in some instances it is the only action that will get results. Until human nature changes, it is unrealistic for management to discard the tool of punishment and fear.

If there are conflicts in human relations, there are also areas of relative peace. Positive motivation has reference to managerial endeavors to maintain peace once it is secured. It is concerned with the incentives or drives that

spur people to work together to obtain desired objectives and goals. It is based on the theory that there are good reasons why people seek harmonious relations with their fellow man. Indeed, it is surprising how well we get along together when we consider how little positive thought we give to harmonious relations. So we have reason to be optimistic about positive motivation because we could do so much better if only we gave as much thought to peace as we give to conflicts.

Steps in handling human relations. Nothing can be accomplished in human relations until an executive knows precisely what the case is with which he is dealing. Knowing what the problem is, is half the solution. In this connection, it is important to gather as much information as is feasible about the grievance, disciplinary case, or the need of positive motivation. Such information gathering is advanced if executives know how to investigate records, listen to others, and observe what is going on. Moreover, they must know how to separate fact from opinion. The powers of appraisal and judgment must be sharp.

Next one must postulate possible alternative solutions to our human relations problems. We should list what seemingly would be desirable to do, and then seek to figure out which is the best alternative. It is sometimes wise to seek the help of other executives or counsel with fellow executives in other companies.

Having decided upon what seems to be the best solution, the next step is to apply it. Yet it is surprising how often executives shrink from doing that. They may not be sure of their solution; they may hesitate to apply discipline; or they are afraid to be positive for fear of being considered "soft." So this type of "apply the solution" is not as simple as it sounds. But in addition, application must also be timed properly. There is a matter of strategy here. For example, to settle a grievance too soon or too late is as bad as a poor settlement. To apply positive motivation at the wrong time is equally ineffective. So an executive not only must apply his solutions but must do so at the proper time, in the proper place, and in the proper manner.

Finally, good human relations requires checking to see if the plans of handling grievances, disciplinary cases, or positive motivation are working out as planned. Only in that way can an executive determine what must be done so that future plans can be made more effective and current plans be as successful as possible.

Principles of human relations. No rules have yet been formulated that will result in complete success in dealing with human relations. All that we have are some rules that will usually help to maximize success and minimize failure. They are not guarantors. Executive judgment is still needed, and risks cannot be eliminated. What is suggested here is therefore only in the way of suggestions. The ideas are not complete. Each executive should keep the ideas he likes and develop his own to add to the list. But for what they are worth, here are some principles that have had the test of experience.

In the handling of grievances, the following are suggested:

1. In dealing with aggrieved individuals, it is unwise to assume that they are ignorant, selfish, unfair, or unreasonable.
2. Display a desire to be fair and show sincere interest in and patience with the aggrieved person.
3. Remember it is easier to lose good will than to build it; so never treat a grievance lightly or as unworthy of attention.
4. Interpret the effect of the past upon the present, since what happens now is always associated with something in the past.
5. In settling grievances, the appearance of fairness is often as important as fairness itself.
6. Take as much time to weigh and evaluate grievances as their importance seems to merit.

In a case of disciplinary action, the following are suggested:

1. Be sure that what seems to be a disciplinary case is really one and not something for which the company is at fault.
2. Recognize individual differences and adjust penalties to fit the individual within limits of policies.
3. Recognize that disciplinary action should be used to prevent future shortcomings and repetitions of undesirable activities; it cannot cure past mistakes.
4. Whenever possible, discipline in private.
5. Add to disciplinary action positive suggestions of what a person can and should do to prevent future penalties.
6. Remember that the attitudes of executives often have as much influence on future behavior as the penalties themselves.

With regard to positive motivation, the following are suggested:

1. Self-interest does not necessarily mean greediness; indeed, intelligent self-interest is a good and reasonable force that should be encouraged.
2. Learn to judge people so that to each the proper positive motives may be applied.
3. Reward people in terms that indicate they were earned and not given to them.
4. Stress nonfinancial goals and prestige symbols of accomplishments because they are coveted as much as financial rewards.
5. Proportion rewards to fit the occasion, the effort, or the difficulty of accomplishment.

6. Rewards must be not only attainable but also related to effort, within a reasonable time.

Effect of Particular Situations and Environments

More and more it is being recognized that the environment in which one moves has much to do with the way he thinks, acts, and feels. This environment is composed of political, social, and psychological as well as economic situations. Thus, it is that a person from a particular part of a community, with a certain type of education, raised under a given standard of living, and holding certain political views will react differently to an executive's request than will one who comes from another set of circumstances. It is for that reason that an executive, for example, thinks differently about promotions than do many of his subordinates. All of which places a great burden on an executive. If he hopes to deal effectively with his subordinates as people, he must know the backgrounds of each of them; he must give due consideration to them; he must be tolerant of them; and he must be forever a student of what makes people behave as they do.

If environment, as well as heredity, determines human behavior, executives must be students of the effects of different environments. For example, if a group of people honestly believe that it is to their advantage to avoid working too hard—to restrict out—disciplinary action won't do much good. We must find out why they hold such ideas and why they cling to them. Only then can we know what must be done, and how, to eradicate the block to cooperation.

Until we are willing to look at the others' opinions with a searchlight of respect and intelligence, we can hope for little progress in human relations. But if we do look upon differing ideas with understanding, with patience, and with tolerance, we shall take an immense step forward in gaining the respect and cooperation of labor. With such cooperation we shall advance the interests of our companies far beyond the reach of our competitors. For although we may advance ahead of others for technological reasons, we can only maintain our advances if we have a cooperative labor force. In the final analysis we cannot forget that it is human beings who provide the brains and the motivation to run the machines of our industrial world. It is the duty of management to install in labor a desire to run these machines with enthusiasm and efficiency.

Some of the material presented was adapted from *Personnel Management* by M. J. Jucius, published by R. D. Irwin, Chicago, 1955.

Developing Educational Personnel[1]

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Although subject matter is of greatest importance in developing a dairy program in colleges and experiment stations, personnel problems and opportunities play a very important part in the success of this program. This applies to all fields of agriculture. The objective of this paper is to discuss some of the factors involved in selecting, training, and developing personnel for educational work in college dairy departments.

People vary in temperament, ability, training, and experience. This is fortunate since many kinds of jobs must be handled. Some people are good leaders; some are good followers. Sometimes we have people who can be both. Every one of us at one time was a beginner, and somebody took a chance on us to help develop us either for a particular objective or an incidental necessity. Some people are outstanding in research, extension work, or teaching. This includes some who make their greatest contribution to developing young men. Only a few may be good supervisors or administrators.

Our responsibilities are to our superiors; our coworkers in other fields; those who work under our supervision, and those with whom we have no official relationship but whose assistance and cooperation we need. Where interests and activities of personnel overlap, personnel problems very frequently develop. Where there is no overlapping of activities, there is less personal difficulty.

Some people continually create "bottlenecks" and are never able to work themselves out of them. Someone must come to their rescue. Others are good at breaking "bottlenecks." Some people are at their best when working with people in a group or under pressure. Others do their best when working alone. These difficulties may involve personnel adjustments as well as financial problems and working conditions.

Administrators and supervisors have duties and obligations, and so also do workers and

[1] Presented at Meeting of Southern Division, American Dairy Science Association, Atlanta, Georgia, February 7, 1956.

employees have rights, privileges, and obligations. As the expression goes, "It is necessary to have both chiefs and Indians." Sometimes we have too many chiefs and not enough Indians; sometimes the reverse.

In agricultural college circles (as well as others) the general practice throughout the country has been to promote to administrative and supervisory positions individuals who are outstanding in their particular fields of work within the organization. Sometimes they have had little or no specific training for supervisory or administrative activities. However, they usually have gained some training in this field through experience. Such persons are generally fortified with good contacts, good judgment, and ability to lead others. Some fields of activity in college training programs feature a maximum of training in methods and procedures.

In handling personnel the factors to consider are personal recognition and titles; attitude toward work; security and morale; encouragement and criticism; delegating and accepting authority; measuring productivity; duties and responsibilities; satisfactory working conditions and facilities, fairness and credit; salaries and promotion; leaves, vacations, and retirement.

It is essential that administrators or management *(4)*:

1. Be genuinely interested in the development of men.
2. Be aware of the abilities and talents of those within its organization.
3. Be able to select those who show promise for further growth and development.
4. Provide opportunities for experience and training which will contribute to self-development.
5. Provide guidance and counsel which will help the individual make the most of these experience opportunities and to arrive at a better understanding of himself and his abilities. These points illustrate how commercial concerns are aware of the problems of personnel and their attitude in developing their employees. Do educational institutions give enough attention to this?

When dealing with people it is important to recognize Deldin's *(1)* ten basic principles of sound human relations.

1. All of us resent domination. 2. We all enjoy a good fight. 3. We are more likely to agree with those we like personally. 4. We are inherent sentimentalists. 5. We all want to feel important. 6. We all want to be "in the know." 7. We like to win out over obstacles. 8. We are all different. 9. We must recognize the inevitability of gradualness. 10. We all want all we can get. Many persons will recognize these characteristics in people. It remains for us to focus our attention on these elementary personnel traits in working with people.

Kathryn Saunders *(6)* reports, "Even the well selected, placed, and adjusted employee not infrequently finds himself subordinated to persons who themselves are not well adjusted. It is a constant source of irritation to him to be subjected to the whims of an executive or supervisor who is sadistic, paranoid, moody, (hypochondriacal), or in some other way seriously maladjusted." Many of us recognize such a personality in our dealings with people. The trouble is, some people look on the other fellow as the one fitting this description.

A well-known dean of agriculture spoke to his administrative and supervisory staff on the subject of "Personal Investment" and asked the following questions:

1. How much does your institution have invested in you?
2. What do you think you are worth to your state?
3. How important is your connection with your institution? Who would come to you for information, if you were not connected with an educational institution?
4. What part does remedial transfer play in handling of personnel?

If you will answer these questions as applied to yourself, you will realize what an administrator is looking for in a college staff.

Inventions and techniques have run ahead of our ability to deal with people. Our leaders fly the Atlantic in a day on a peace mission, but what do they do when they get there? As General Omar Bradley said recently, "The world has achieved brilliance without wisdom, power without conscience. Ours is a world of nuclear giants and ethical infants. We know more about war than we know about peace; more about killing than we know about living."

Teachers

Teachers succeed or fail according to their background of training and experience, ability to work with people, desire to teach and to study their job, and examples of good teaching. In addition they must grow with experience, develop interesting or inspiring techniques, receive support of administrators and have desirable personal attitudes. Students in a class are not good judges of teachers. It takes years of experience after leaving school for a person to accurately appraise former teachers.

Research Personnel

"The individual in research is much more important than impressive buildings, modern equipment, or an unlimited budget," according to William B. Given *(3)*. It is important that motivation and characteristics of the research worker be understood and an intelligent attempt be made to satisfy his desires. Laboratories and libraries have an important place in research work and should be among the least regimented places to work. A research worker

needs freedom of thought and action, and any attempt to restrict his searching study is frustrating.

Overstreet *(5)* states: "A different kind of contribution is being made by those who set up research projects—by actual experiment,"—about how people affect one another in group situations. Unresolved personal problems commonly underlie behaviors that make people get on other people's nerves.

Dr. Ralph W. Sockman *(7)* stated, "If we are performing an experiment in a chemical laboratory, we can make our analyses and wait until the data are compiled before we make up our minds. But if you are the head of a firm and have to make an appointment to a position, you consider the various candidates. From their credentials you know what other people think about them. But there comes a point where you must make up your mind, entrust one of the applicants with the job and see if he makes good. In dealing with facts we can often be sure before we commit ourselves. But in dealing with persons we must commit ourselves before we can be sure." Many administrators recognize the personal responsibility they must assume in arriving at decisions in selecting personnel.

Beaumont *(2)* lists eleven subjects on the psychology of personnel as follows: Understanding employees; analyzing jobs; selecting employees; training employees; working conditions; the workers' health; promoting safety; supervision; merit rating; providing incentives; occupational adjustment. Administrators and supervisors gain experience along these lines. There should be adequate provision somewhere in training programs to take advantage of the experience of others and studies along these lines.

The results of a survey with dairy departments of state agricultural colleges by the author are as follows:

1. Staff replacements due to resignation varied considerably from excellent to average; however, a shortage of research and teaching "assistants" was quite evident. More students could be trained with existing personnel, equipment, and other facilities under existing teaching programs. At the present time it is not a problem to place students on a job.

2. Teacher-student problems and public contacts for staff were rated very good.

3. Programs for graduate study rated better than did undergraduate programs. Such programs need to be continually revised to meet new problems.

4. The ability of staff members to work together and coordination of teaching and research are still problems in some departments. Subject matter information is running ahead of plans, ability, and procedure for personnel to work together harmoniously.

5. On points involving financing, few were rated as excellent. Operation budgets varied from excellent to poor. Policies in marketing dairy products were favorable and definitely better than dairy farm financing.

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(2) Beaumont, Henry. *The Psychology of Personnel.*
(3) Given, Wm. B., Jr. *Reaching Out in Management*, p. 86.
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(5) Overstreet, Harry and Bonaro. *The Mind Alive*, p. 250.
(6) Saunders, Kathryn. The Psychology of Employee Counseling: An Analysis of Its Functions and Practices. Thesis, Univ. of Tennessee. p. 348.
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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

341. Prévention et Traitement des Mannites (Prevention and Treatment of Mastitis). R. RICHOU and G. THIEULIN. Le Lait, **36**: 1. 1956.

This short review is based primarily on the experiences and observations of the authors. They recommend general cleanliness and normal care of the animals; use of a simple test for early detection of mastitis; elimination of "incurables"; use of chemicals and antibiotics for specific treatment; and the use, when possible, of vaccines. A.W. Rudnick, Jr.

342. Control and treatment of mastitis in cows. F. E. NELSON, Iowa State Coll., Ames. Milk Plant Monthly, **45**, 4: 27. 1956.

Milk yield may be reduced 20% by mastitis, which usually is caused by infectious bacteria rather than by mechanical injury. In extreme cases part of the udder is destroyed and may actually slough off. More frequently, tissue which previously produced milk may be replaced by nonproductive scar tissue. The infection almost always is confined to the udder and only rarely does a cow die from mastitis. Many different species of bacteria may cause mastitis. *Streptococcus agalactiae* is the most common and *Staphylococcus* sp. the second most important cause of the disease. Early detection, prevention of udder injury, and proper treatment of all cases are essential for reduction of the losses due to mastitis. C. J. Babcock

343. The size of fat globules in the milk of diseased cows. J. O. L. KING, Univ. Liverpool Vet. Field Sta., Neston, Wirral, England. J. Dairy Research, **23**, 1: 105. 1956.

The effects of temperature rise in milking cows due to disease upon milk yield, fat content, and fat globule size were determined from studies involving 31 animals. The effect of sudden environmental changes upon fat globule size was investigated, e.g., delivery to and treatment in a veterinary clinic. Such treatments did not influence percentage of fat or size of the fat globules. In a group of animals in which rectal temperatures were above 102.4° F. for at least two consecutive milkings from which the milk was composited, the elevated body temperatures adversely affected yield. The fat content of such milk was increased and the fat globules were larger. J. D. Donker

BUTTER

344. Detection of adulteration of butter with foreign fats by examination of the sterols. P. C. DEN HERDER. Neth. Milk Dairy J., **9**: 261. 1955.

A method presented for the detection of adulteration of butter with margarine is based on a microscopical examination of the crystalline sterols. A procedure for the determination of the melting point of the acetates also is presented. W. C. van der Zant

345. The effect of the copper content on the development of flavor defects in cold-stored butter. C. I. KRUISHEER and B. M. KROL. Neth. Milk Dairy J., **9**: 173. 1955.

Seventy-one samples of summer butter and 54 samples of winter butter were examined before and after storage for 8-12 mo. at –20° C. for defects such as greasy, fishy, and train-oil. All of the butter was unsalted and made from ripened cream. Tocopherol, carotene, vitamin A, copper content, and pH were also determined.

In summer butters, only samples with a copper content of less than 50 γ per kg. did not show defects after cold storage. Samples of summer butter containing more than 70 γ copper per kg. had off-flavors after cold storage. With winter butter the relation between copper content and keeping quality was less clear. Samples of winter butter with high copper content showed fewer defects. The authors suggest a relation between the higher natural copper content in winter butter and the greater resistance to oxidation. W. C. van der Zant

346. The total tocopherol (vitamin E) content of Dutch butter. C. I. KRUISHEER. Neth. Milk Dairy J., **9**: 275. 1955.

Samples of butter from 18 factories in the Netherlands were examined monthly during 1 yr. for total tocopherol, carotene, vitamin A, and copper. A part of the sample was stored at –20° C. for 8 to 12 mo. to observe the development of flavor defects caused by oxidation.

The tocopherol content of butter was as follows: whole year, 8-34 (mean 20) γ/g butter, 10-40 (mean 24) γ/g butterfat; pasture period, 20-34 (mean 24) γ/g butter, 23-40 (mean 29) γ/g butterfat; stall feeding period, 8-21 (mean 15) γ/g butter, 10-25 (mean 18) γ/g butterfat. There was no appreciable correlation between the tocopherol and the carotene contents from May to August; in the other months the correlation was positive with a fair significance. The relation between the tocopherol and the vitamin A content was of an irregular character. On a statistical basis a distinct antioxidant effect of tocopherol in butter seemed unlikely. There was some indication that butter manufactured during the pasture period had a greater tendency to develop oxidation defects.

W. C. van der Zant

347. Loss of vitamin A potency during the preparation of ghee from milk. H. S. Patel and B. M. Patel. Indian J. Dairy Sci., **8**: 53. 1955.

The average loss (14 trials) of carotene and vitamin A in the preparation of ghee from cow's milk was 21 and 30%, respectively. About 20% of the vitamin A was lost when ghee was made from buffalo milk. The method used in the manufacture of ghee did not affect the losses of carotene and vitamin A.

W. C. van der Zant

CHEESE

348. Antibiotic cuts defects in processed cheese. Anon. Food Eng., **28**, 3: 90. 1956.

Experience with the use of the antibiotic, nisin, in the control of clostridia in processed cheese is growing rapidly. Characteristics of nisin make it an ideal inhibitor for use in foodstuffs. Highly active preparations have been produced commercially. These preparations are transferred to skimmilk cheese curd by a process that renders the nisin extremely stable. The special cheese contains approximately 10,000 Reading Units (RU) nisin per g. and is incorporated with other cheese during processing at the rate of 1½-2% to give a concentration in the processed cheese of 150-200 RU/g.

The antibiotic is not greatly affected by melting temperatures up to 204° F. However, its efficacy in cheese may be influenced by pH, type of emulsifier, and bacterial flora of the cheese. Development of a process for manufacture of pure nisin is now beyond the pilot plant stage. It is expected that the product will be released to food processors in the near future.

T. J. Claydon

349. Manufacture of cheese. N. Kraft and P. J. Ward (assignors to Kraft Foods Co.). U. S. Patent 2,743,186. 12 claims. Apr. 24, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 4: 823. 1956.

In the manufacture of ripened cheese, the drained curd is immersed in a solution of an edible acid at a temperature between 90° and 120° F.

R. Whitaker

350. Manufacture and preservation of cheese. A. T. R. Mattick and A. Hirsch (assignors to National Research Development Corp.). U. S. Patent 2,744,827. 8 claims. May 8, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 2: 368. 1956.

Cheese is protected from spoilage by adding nisin and nisin-producing bacterial cultures.

R. Whitaker

351. Storage racks for cheeses. J. G. R. Eales and E. Hale (assignors to Co-Operative Wholesale Society Ltd.). U. S. Patent 2,744,325. 2 claims. May 8, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 2: 237. 1956.

A device for turning cheese upside down during curing.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

352. Method of producing egg substitutes for use in production of sponges, cakes, and similar baked goods. K. Kumetat (assignor to Commonwealth Scientific and Industrial Research Organization). U. S. Patent 2,739,898. 15 claims. Mar. 27, 1956. Offic. Gaz. U. S. Pat. Office, 704, 4: 803. 1956.

An egg substitute for use in baked goods, consisting of a mixture of skimmilk, casein, and casein with small amounts of lactalbumen and lactoglobulin and a calcium sequestering agent. A final pH above 7 is obtained by neutralization with an alkali. At no time is the temp. allowed to exceed 60° C.

R. Whitaker

353. Protein from aerated sewage and whey. O. Rahn (assignor of ½ to Robert V. Morse). U. S. Patent 2,742,359. 5 claims. Apr. 17, 1956. Offic. Gaz. U. S. Pat. Office, 705, 3: 610. 1956.

An animal feed made from aerated sewage sludge and whey solids. The product is rendered sterile by fermenting the whey to produce a high degree of acidity prior to sterilization of the mixture.

R. Whitaker

354. Preservation of Indian milk sweets. Some preliminary studies on Shrikhand Wadi and milk burfee. W. B. Date and D. S. Bhatia. Indian J. Dairy Sci., **8**: 61. 1955.

The keeping quality of Shrikhand wadi was low because of the development of rancid flavors. The moisture content and acidity of this product affected the keeping quality to a great extent. Burfee could be stored for 6 mo. if the moisture content ranged from 5 to 9%. Incorporation of an antioxidant prolonged the shelf-life. Gas-packing did not improve the keeping quality.

W. C. van der Zant

355. Over de oorzaken van verhogingen van het kiemgetal van verstuivings melk poeder (Causes of increased plate counts of spray

dried milk powder). TH. E. GALESLOOT. Neth. Milk Dairy J., **9**: 194. 1955.

S. durans and *S. thermophilus* were nearly always found with increased plate counts. *M. caseolyticus* was sometimes found. A smaller increase in bacterial counts could also be caused by *S. bovis, S. faecalis,* and micrococci. In a few samples *B. cereus* was the cause of high plate counts. A comparison was made between plate counts at 37° and 30° C. The differences between the counts of 37° and 30° C. were caused by *M. lacticum,* which did not grow at 37° C. W. C. van der Zant

356. Process for producing a water-soluble casein. D. F. WAUGH (assignor to National Dairy Research Laboratories, Inc.). U. S. Patent 2,744,891. 8 claims. May 8, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 2: 380. 1956.

A water-soluble alkali metal caseinate is made from skimmilk by adjusting the calcium concentration to 0.05 to 0.1 *M* at not over 60° C., removing the calcium caseinate thus formed, and treating it with a sequestering agent to reduce the calcium content to below 0.2% (dry basis). The calcium is removed by dialysis or by precipitation of an insoluble calcium salt, maintaining the pH in a range from 5.3 to 6.5.
R. Whitaker

357. Proteinaceous composition and method of making the same. J. I. WAGNER, B. HABIGHURST, and M. G. TERRY, JR. (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,744,890. 5 claims. May 8, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 2: 380. 1956.

A dressing for burns and other skin injuries consisting of nonantigenic coagulable casein from milk, with about 0.25 to 1% of an age thickening inhibitory substance such as urea, cysteine, methinone, and hydrolyzed protein.
R. Whitaker

DAIRY BACTERIOLOGY

358. The stability of Lactobacillus strains. G. H. G. DAVIS and A. C. HAYWARD, Univ. of Birmingham, England. J. Gen. Microbiol., 13, 3: 533. 1955.

Studies were made on the "stabilizing effects" of repeated culture on pure strains of *Lactobacillus* in which changes from the heterofermentative to the homofermentative type of organism have presumably been reported. The authors conclude that no evidence is available suggesting that such gross variations do occur and that such changes observed in cultures may be explained on the basis of inadequate initial purification and subsequent contamination of strains of different physiological types.
J. J. Jezeski

359. A survey of physically active organic infusoricidal compounds and their soluble derivatives with special reference to their action on the rumen microbial system. J. M. EADIE, S. O. MANN, and A. E. OXFORD, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., 14, 1: 122. 1956.

A variety of substances insoluble or only slightly soluble in water including hydrocarbons, non-nitrogenous alcohols, non-nitrogenous ethers, indol derivatives, glucosides, glucuronides and monoalkyl succinates exhibited a lethal action against living ciliates from the rumen, as determined by microscopic examination of washed suspensions of living protozoa obtained from fistulated sheep. Toxicities were observed at or near saturation concentrations at neutral pH. The terpene alcohols and their soluble glucosides and the acid succinates were particularly toxic; different effects were observed on the protozoa suspensions alone as compared with protozoa in whole rumen liquor containing bacteria. Partial explanation of the toxicities was based on the presence of esterase of protozoan or bacterial origin. Considerable numbers of tributyrin-hydrolyzing bacteria were detected in rumen contents. J. J. Jezeski

360. Some factors affecting the formation of iodophilic polysaccharide in group D streptococci from the rumen. P. N. HOBSON and S. O. MANN, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., 13, 3: 420. 1955.

Seven of 25 cultures of alpha hemolytic group D streptococci previously isolated from the rumens of calves produced an intracellular iodophilic polysaccharide from a variety of starches and other carbohydrate materials. Not all carbohydrate materials tested gave rise to the production of this substance. Production of the polysaccharide occurred on agar media, either aerobically or anaerobically to a greater extent than in liquid media. The polysaccharide was produced only from maltose when the pH value was somewhere between pH 5 and 6. On the basis of a variety of tests, it would appear that this bacterial polysaccharide did not fit any of the usual specifications of starch, amylose, amylopectin, or glycogen. J. J. Jezeski

361. Efficacy of chlorine, a quaternary ammonium compound and a new disinfectant (chlorhexidine) in teat cup decontamination. J. H. STEWART, G. R. SPENCER, J. LASMANIS, and D. M. HOLLIDAY, Coll. Vet. Med., State Coll. of Wash., Pullman. J. Milk and Food Technol., 18, 89. 1956.

Chlorine, a quaternary ammonium compound, and chlorhexidine (a new disinfectant) were compared for activity against bacteria largely responsible for bovine mastitis. Chlorine was the least effective in germicidal activity, especially in the presence of milk. Chlorhexidine was superior to chlorine in its germicidal properties in the presence of organic matter and possessed a wider bacterial spectrum.

Chlorhexidine showed a higher bacteriostatic effect at comparable concentration of sodium

hyopchlorite. *In vitro* the new disinfectant exhibited more bactericidal and bacteriostatic effect than the quaternary against the test organisms studied. H. H. Weiser

362. Recent developments in food uses for antibiotics. C. L. Wrenshall and J. R. McMahan, Chas. Pfizer & Co., Brooklyn, N. Y. J. Milk and Food Technol., 19, 104. 1956.

Emphasis has been placed on the practical applications of antibiotics in the preservation of foods. Government regulatory policies are considered in regard to present and future uses of antibiotics in food products.

H. H. Weiser

363. Studies on the micrococci in milk. I. Incidence and distribution. V. Lakshminarasim and K. V. Iya. Indian J. Dairy Sci., **8**: 67. 1955.

Micrococci constituted approx. 67 to 77% of the total bacterial content of farm milk produced during different seasons of the year. Of these micrococci, 9-20% were thermoduric, and the micrococci constituted 63 to 82% of the total thermoduric flora. Species which produced transparent or white growth on agar predominated over the pigment forming types. Representative micrococci were isolated and their characteristics and classification discussed.

W. C. van der Zant

364. Studies on the micrococci in milk. II. Biochemical activities. V. Lakshminarasim and K. K. Iya. Indian J. Dairy Sci., **8**: 108. 1955.

Growth rate, acid production, and proteolytic activity of *M. caseolyticus, M. flavus, M. curantiacus,* and *M. rhodochrous* were studied. The influence of the growth medium, pH, incubation temperature, and light on the pigment production was also studied. Pigment production was pronounced in tryptone-glucose-agar with 1% skimmilk at pH 7.0-7.2 at 30° C. Certain characteristics of the pigments are discussed.

W. C. van der Zant

365. Het aantonen van penicilline in melk- and zuivel-production (The detection of penicillin in milk and dairy products). Th. E. Galesloot. Neth. Milk Dairy J., **9**: 158. 1955.

The following methods were developed: Milk: a 30-ml. sample is divided equally into 2 culture tubes and heated at 80° C. for 10 min. Penicillinase is added to one tube, and both tubes are held at 37° C. for 0.5 hr; then 0.1 ml. of a *S. thermophilus* culture (16-24 hr., 37° C.) and 0.4 ml. of a methylene blue solution are added followed by incubation at 45° C. Differences in reduction time are considered as proof of the presence of penicillin. Acid dairy products (buttermilk, sour cream, starters, yoghurt): a 30-ml. aliquot of the product is neutralized with NaOH (10%) to pH 6.8, and the test completed as for milk. A sensitive extraction method also was developed for penicillin, and the stability of penicillin in acid dairy products and cheese was investigated. Penicillin (0.05 I.U. per ml.) was added to milks with pH values of 4.5, 5.0, and 5.5, and the milks were stored at 2° and 20° C. At pH 4.5 no penicillin activity was found after 5 days; at pH 5.0 and pH 5.5 after 7 and 8 days, respectively.

W. C. van der Zant

366. Over de groei van aromabacterien in zuursels (Growth of flavor forming bacteria in starters). K. Holwerda. Neth. Milk Dairy J., **9**: 204. 1955.

The logarithmic growth curve of the aroma bacteria usually showed an upward trend when the number of streptococci had reached a certain level. At pH 4.8 the growth of lactic acid and aroma bacteria stopped.

W. C. van der Zant

367. Een onderzoek van penicilline-bevattende melk (Examination of penicillin-containing milk). A. D. Adamse. Neth. Milk Dairy J., **9**: 121. 1955.

With lactic acid cultures the limit of sensitivity was 0.05 IU per ml. of milk. At a level of 0.3 IU per ml. no formation of lactic acid occurred. The formation of diacetyl was considerably reduced at 0.25 IU per ml. Yoghurt cultures were 10 times as sensitive as a normal starter. Pasteurization did not affect the influence of penicillin. In very small concentrations (0.001 IU/ml) penicillin had a stimulating effect on lactic acid bacteria in milk.

W. C. van der Zant

368. De vorming van zwavelwater stof in Goudse kaas, veroorzaakt door melkzuur bacterien (Lactic acid bacteria producing hydrogen sulfide in Gouda cheese). J. W. Pette. Neth. Milk Dairy J., 291. 1955.

Bacteria responsible for the production of H_2S in Gouda cheese were isolated with a medium consisting of 3% Witte peptone, or 1% Witte peptone and 0.1% cysteine hydrochloride. The pH of the cheeses with the defect was usually too high and the salt content too low. A detailed description of the biochemical characteristics of the spoilage organisms is presented. The author proposed the name *S. faecalis* var. *malodoratus* for the organism responsible for the defect. The defect could be prevented by using less water during cooking, and by keeping the pH of the cheese at an age of 6 weeks below 5.4. W. C. van der Zant

369. Influence du chauffage du lait sur le dévelopment des bactéries; Croissance des bactéries lactiques dans des laits chauffés à des températures variables. (Influence of heating of milk on the development of bacteria; Growth of some lactic bacteria in milks heated at various temperatures. J. E. Auclair and A. Portmann, Cent. Sta. of Microbiol. and

Dairy Research, Jouy-en-Josas. Le Lait, **36**: 145. 1956.

Heat treatment of the milk caused marked variation in the amount of acid developed by pure cultures, and optimum heat treatment varied according to the test organisms used. Maximum acid development of *S. agalactiae* 1B occurred in milk heated to 80° C.; best acid development of *S. lactis* X13 occurred in milk heated to 105° C.; heat treatment had little effect on acid production of *S. lactis* 7M10; acid production of *S. cremoris* 1P5 was at a peak at 70° and 110° C., the latter being the higher; two peaks of acid production were also exhibited by *S. thermophilus* TJ, one at 65° C. and the other at 120° C., again the latter being the higher; little acid was produced by *L. lactis* O37 in milks heated under 100° C., but good acid production occurred in all samples heated over 105° C. Acid production in milk from various cows differed in degree but followed the same general curves with regard to acid production.

The authors attempt to explain the double acid production peak of some of the organisms by the destruction of lactinins in the temperature range of 70° to 90° C.

A. W. Rudnick, Jr.

370. Detection of lactic starter inhibitors using 2,3,5-triphenyltetrazolium chloride. C. E. Neal and H. E. Calbert, Univ. of Wis., Madison. Milk Prod. J., **47**, 3: 14. 1956.

A comparatively simple biological test has been devised for the detection of antibiotics in milk. The test employs an actively growing lactic culture and 2,3,5-triphenyltetrazolium chloride (TTC). This test can be completed in 2½ hr. and is very sensitive. The level of antibiotic concentration detectable is determined by the type of culture used in the test. Directions for the test are presented.

Advantages of the TTC test over other methods available for detection of inhibitory substances are discussed:

a) The same equipment used for the methylene blue reduction test can be used.

b) The test is sensitive to all the antibiotics commonly used to treat mastitis.

c) The test will detect inhibitory substances other than antibiotics which may cause difficulty in the manufacture of dairy products.

d) The test can be performed by a person familiar with simple laboratory technique.

e) The cost of materials for the test is very small.

J. J. Janzen

DAIRY CHEMISTRY

371. Determination of 6-ethoxy-1,2 -dihydro-2,2,4-trimethylquinoline in biological materials. E. M. Bickoff, J. Guggolz, A. L. Livingston, and C. R. Thompson, Western Utilization Research Br., Agr. Research Ser., U.S.D.A., Albany 10, Calif. Analyt. Chem., **28**, 3: 376. 1956.

The antioxidant, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, is commonly used for stabilizing carotene in dehydrated alfalfa meal. Feeding tests have shown that its toxicity is low and alfalfa meal treated with the antioxidant for use in poultry feeds is now shipped in interstate commerce. This study describes a method of assay for the antioxidant, sufficiently sensitive to permit its detection in animal tissues, eggs, milk, butter, and treated alfalfa meal. The lower detection limit of the method is about 0.01 p.p.m. of the antioxidant in a solvent solution.

B. H. Webb

372. Colorimetric method for determination of sugars and related substances. M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, Div. of Biochem., Univ. of Minn, St. Paul. Analyt. Chem., **28**, 3: 350. 1956.

An orange-yellow color is given by simple sugars, oligosaccharides, polysaccharides, and their derivatives, when they are treated with phenol and concentrated sulfuric acid. The reaction is sensitive and the color is stable. By use of this phenol-sulfuric acid reaction, a method has been developed to determine submicro amounts of sugars and related substances. The amount of color produced at a constant phenol concentration is proportional to the amount of sugar present. Standard curves are obtained by plotting the sugar concentration against the absorbence which is measured by the spectrophotometer. Reagents are inexpensive, stable, and readily available. The method has been applied to analysis of hydrolysates of oligosaccharides and to the determination of the amount of sugar in urine and blood.

B. H. Webb

373. Vitamin assay. Microbiological and chick assay of vitamin B_{12} activity in feed supplements and other natural products. W. L. Williams, A. V. Stiffey, and T. H. Jukes, Nutrition and Physiol. Sect., Research Div., American Cyanamid Co., Pearl River, N. Y.

Recent modifications and improvements of the *Ochromonas* and *E. coli* methods, as well as the details of a chick growth method for determining vitamin B_{12}, are presented. On the basis of comparative determinations of vitamin B_{12} activity in 40 samples, it is concluded that pseudocobalamins—i.e., substances which promote the growth of certain microorganisms but not the growth of animals—do not occur commonly to a large extent in vitamin B_{12} feed supplements. The USP *L. leichmannii* method will give reliable results with most vitamin B_{12} feed supplements, but it is advisable to make at least one test with both the *Ochromonas* and chick methods. Certain fermentation materials contain substances which are inhibitory to chick growth, so that the chick assay method cannot be used in such cases.

S. Patton

374. Enzyme assay. Modified milk-clotting procedure for determination of papain activity. O. Gawron and F. Draus, Dept. of Chem.,

Duquesne Univ., Pittsburgh, Pa. J. Agr. Food Chem., 4: 349. 1956.

A technically simple and rapid method for estimating enzyme activity was desired for investigation of the chromatographic behavior of papain and chymopapain on ion exchange columns. A modified milk-clotting procedure, using cysteine as the activator, gives a linear relationship between inverse time of clotting and enzyme concentration over a fifteenfold range of enzyme concentration. Ammonium sulfate and sodium chloride inhibit enzyme activity, and determination of activity in their presence necessitates use of correction factors. The method is rapid and precise (2%) and suitable for routine determinations of enzyme activity in both absence and presence of inhibitory salts. S. Patton

375. Préparation de présure cristallisée a partir d'estomacs de veaux (Caillettes) (Preparation of crystallized rennet from calves' stomachs). C. Alais, Central Station of Dairy Microbiol. and Research, Jouy-en-Josas. Le Lait, 36: 26. 1956.

The method described is a simplification of that of Berridge; however, instead of using commercial rennet extract as a starting point these authors use calves' stomachs. They described a standard method to measure activity. From electrophoretic patterns and photomicrographs of the crystals it was concluded that the material was homogeneous. Activity of this preparation was somewhat higher than that of Berridge. It was noted also that activity of the preparation increased markedly when suspended in a phosphate solution rather than water. Such solutions were stable, and lost only about 17% of their activity in four months.
A. W. Rudnick, Jr.

376. La purification de l'acide lactique par extraction (Purification of lactic acid by extraction). G. Genin, Le Lait, 36: 34. 1956.

A brief review of the various methods of purifying commercial lactic acid.
A. W. Rudnick, Jr.

377. Color reaction of amino acids with alloxan, isatin, and ninhydrin in circular paper chromatography. A. Saifer and I. Oreskes, Isaac Albert Research Inst. and Dept. of Phys. Chem., Jewish Chronic Dis. Hospital, Brooklyn, N. Y. Anal. Chem., **28**, 4: 501. 1956.

Color reactions of 51 amino acids and their sensitivities were determined as spot tests on filter paper with alloxan, isatin, and ninhydrin. R_f values for these amino acids were obtained with the circular chromatographic method using phenol- and butanol–acetic acid as developing solvents. The nature of these color reactions for amino acids and their possible application to amino acid mixtures are discussed.
B. H. Webb

378. Determination of triphosphate in commercial triphosphate and detergents built with triphosphate. H. J. Weiser, Jr., Procter & Gamble Co., Cincinnati, Ohio. Anal. Chem., **28**, 4: 477. 1956.

Tris(ethylenediamine) cobalt(III) ion [$Co(en)_3^{+++}$] has been shown to be a precipitant for triphosphate ($P_3O_{10}^{-5}$) ion in an acid solution. Based on this reaction, a method for determination of triphosphate was developed which was free from interference of other phosphates, sulfate, and silicate. The determination can be run in 2 hr. and is applicable to commercial triphosphate and triphosphate built detergent samples.
B. H. Webb

379. Detection and paper chromatography of sugars and sugar phosphates in picric acid system. H. S. Loring, L. W. Levy, and L. K. Moss, Dept. of Chem. and Sch. of Med., Stanford Univ., Calif. Anal. Chem., **28**, 4: 539. 1956.

Paper chromatography with the tert-butyl alcohol-picric acid-water solvent system has been limited to sugar phosphates. Its application to sugars in general has now been made possible by the development of a simple spray technique for their detection on paper chromatograms. The reaction involves the reduction of alkaline picrate to picramic acid or other reddish-brown products. Among the sugars examined were galactose, glucose, and lactose. R_f and R_{ribose} values are reported. B. H. Webb

380. Electrophoresis cells modified for determination of labeled protein fractions. R. E. Hein, R. E. Clegg, T. J. Clark, and R. H. McFarland, Kan. State Coll., Manhattan. Anal. Chem., **28**, 4: 544. 1956.

A method for observing the amount of certain radioactive isotopes, such as P^{32}, Ca^{45}, S^{35}, and C^{14}, incorporated in a particular protein fraction has been developed. The ascending arm of the center cell of a Tiselius electrophoretic apparatus is modified so that the radioactivity associated with the labeled protein can be measured as the protein fronts migrate past the observation point. The electrophoretic pattern, protein mobilities, and concentration of the separated protein fractions are not affected by this modification. B. H. Webb

381. Phospholipids in milk. I. A method for the estimation of lecithin, cephalin, and sphingomyelin in milk. B. S. Baliga and K. P. Basu. Indian J. Dairy Sci., **8**: 119. 1955.

A detailed description of the methods for the determination of lecithin, cephalin, and sphingomyelin in milk is presented.
W. C. van der Zant

382. Het anorganische sulfaat in rundermelk, -biest, en -bloed (The inorganic sulfate content of milk, colostrum and blood of cows). E. E. van Koetsveld. Neth. Milk Dairy J., **9**: 165. 1955.

A detailed description is presented of a photometric procedure for determining inorganic sulfate in milk and blood. Milk of cows in full lactation contained 10 mg. inorganic sulfate per 100 g., and that of animals in advanced lactation as high as 14 mg. per 100 g. The values for colostrum varied from 0 to 9 mg. per 100 g. Blood serum had an average value of 14.7 mg. per 100 g. Human milk gave values of 0.5 to 2.5 mg. per 100 g., which is lower than in cow's milk. W. C. van der Zant

383. The composition of Dutch butterfat. I. Seasonal variations in the unsaturated fatty acid composition of butterfat. J. STADHOUDERS and H. MULDER. Neth. Milk Dairy J., **9**: 182. 1955.

Spectrophotometric determination of polyunsaturated fatty acids in butterfat showed the presence of conjugated dienoic acids, conjugated trienoic acids, and traces of tetraenoic acids. After isomerization of the fatty acids, the conjugated fatty acids increased considerably. Minor absorption peaks at 348 and 375 mμ might indicate the presence of pentaenoic and hexaenoic acids. Seasonal variations of conjugated dienoic acids ran parallel with the changes in iodine values. The isomerized nonconjugated dienoic acids and the iodine values, however, were negatively correlated. The trienoic acids showed before and after isomerization only a small difference between summer and winter samples. The content of the oleic acid and the iodine value were closely correlated.
W. C. van der Zant

384. Determination of the concentration of calcium ions in milk ultrafiltrate. W. SMEETS. Neth. Milk Dairy J., **9**: 249. 1955.

Four methods are presented for the determination of Ca^{++} in milk ultrafiltrate. The average concentration of Ca^{++} (40 ultrafiltrates) was 2.75 ± 0.39 mmol/l (11 ± 1.6 mg. %). The total Ca content averaged 8.68 ± 1.23 mmol/l (34.7 ± 4.9 mg. %). About 35% was present in the ionic state, 55% was bound to citrate, and 10% bound to phosphate. Milk with added Ca became unstable when the concentration of Ca ions exceeded 4 mmol/l (16 mg. %). The "Utrecht abnormality" of milk probably is caused by an increased concentration of Ca ions. W. C. van der Zant

385. Vitamin determination. Rapid estimation of vitamin A using a surface active agent. E. T. GADE and J. D. KADLEC, The Borden Co., Elgin, Ill. J. Agr. Food Chem., **4**: 426. 1956.

An improved procedure employs a single-step extraction for determining naturally occurring vitamin A in chicken livers, synthetic vitamin A in fortified powdered formulas for infants, and stabilized vitamin A in animal feed supplements. Stabilized vitamin A is determined in the presence of N,N′-diphenyl-p-phenylenediamine by a simple modification of the procedure. The vitamin A is solubilized in the test material with the surfactant and extracted with a mixed solvent. Twenty-five min. is required for one extraction and several may be run simultaneously. The procedure gives good recovery replication, and results comparable to those obtained by saponification.
S. Patton

DAIRY ENGINEERING

386. Unit operation in chemical engineering. The important developments of 1955. Ind. and Eng. Chem., **48**, 3: 467. 1956.

This special edition of Ind. and Eng. Chem. includes 93 pages of reviews in unit operations in chemical engineering and in the fundamentals of chemical engineering. Among others, there are papers on: centrifugation, crystallization, drying, evaporation, and ion exchange as they apply to the broad area of chemical engineering. B. H. Webb

387. The uses of silicones in the dairy and food processing industries. C. W. TODD. Dow Corning Corp., Midland, Mich. J. Milk and Food Technol., **19**, 94. 1956.

Silicones are gaining wide acceptance in dairy and food processing plants. Their usefulness in controlling unwanted foam, preventing adhesion in processing or packaging, their heat stability, moisture resistant paints, remarkable electrical insulation material, and finally as a scratch resistant coating for glassware are a few desirable characteristics of silicones.

The low toxicity of the specific silicones insures their safety as harmless material to be used in the food industry. H. H. Weiser

388. Insulating today's cold room. C. O. DAVIS, JR. Milk Plant Monthly, **45**, 4: 22, 1956.

Dow's styrofoam is an expanded polystyrene plastic foam featuring resistance to moisture, light weight, low thermal conductivity, good structural strength, and freedom from odor. The light weight of styrofoam, about ¼ the weight of cork, combined with the fact that it is available in lengths up to 9 ft., results in savings in insulation costs. It is produced in widths up to 12 in. and in 1 in. through 5-in. thickness. It may be applied to masonry walls, wood walls, floors and ceilings, self supported partitions, concrete floors and ceilings, suspended ceilings, and insulated roofs.
C. J. Babcock

389. Automation for the present and the future in the dairy industry. D. MERLIN, Milk Dealer, **45**, 6: 38. 1956

Automation is the adaption of "brain type" machines to control the "work-type" machines. It is most applicable to industries that handle products mainly in the fluid or semi-fluid state, process or manufacture products which enjoy a relatively high and constant demand, and make

few model or product changes. The dairy industry as a whole can meet these criteria. Its application can be helpful in two ways. It can reduce man power requirements and thus directly reduce processing costs, or improve product quality control by eliminating losses due to spoilage, nonuniformity, and processing wastes. The dairy plant of the future is described.
C. J. Babcock

390. Automation for the present and the future in the dairy industry. D. MERLIN. Milk Prod. J., **47**, 3: 17. 1956.

Most problems associated with automation are not technological but economic, social, and managerial. The dairy industry is well along the road toward automation with processes such as; (1) HTST units with flow diversion valves, (2) continuous buttermaking, (3) ice cream making—practically continuous, and (4) cheese making—partly mechanized. Automatic milk packaging and milk carton sealing are a reality.

Other developments involving greater mechanization and leading toward automation include bulk tank pick-up, in-place cleaning of pipe lines, automatic decrating and crating machines, automatic case stackers, and conveyors of all types. J. J. Janzen

391. Apparatus for continuous production of filled and sealed tetrahedral packages of paper or the like. R. RAUSING (assignor to Hermorion Ltd.). U. S. Patent 2,741,079. 6 claims. Apr. 10, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 2: 258. 1956.

The equipment for forming and filling the tetra-pak paper milk container is described.
R. Whitaker

392. Tank construction for cooling liquids. L. BUEHLER, JR. (assignor to The Creamery Package Mfg. Co.). U. S. Patent 2,740,269. 6 claims. April 3, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 1: 47. 1956.

A jacketed vat for cooling milk or other fluids having a built-in refrigeration unit employing dichlorodifluoromethane as a refrigerant. R. Whitaker

393. Refrigerated trucks. R. H. DIETERICH, National Dairy Products Co., New York, N. Y. Milk Dealer, **45**, 6: 46. 1956.

A conservative approach for refrigerating retail trucks is to install on one wheel housing of the truck three eutectic plates mounted side by side and surrounded by a housing. There would be an air intake at the bottom and a circulating fan at the top. Refrigeration would be supplied by means of a self-contained 1-ton compressor which would operate only while the truck is in the garage but would permit taking advantage of preloading refrigeration by a means of a plug-in unit while the engine is not operating. C. J. Babcock

394. Multi-stage vacreation halves steam use. J. K. SCOTT, Dairy Research Inst., Palmerston North, New Zealand. Food Eng., **28**, 3: 74. 1956.

In butter manufacture, deodorizing cream with the Vacreator normally requires large amounts of steam. Greater efficiency is achieved by incorporating 2 or more deodorizing units, with the steam flow countercurrent to the cream. In this arrangement, partially tainted steam can remove some volatile substances from fresh cream, because concentration in the latter is high enough to establish a new equilibrium. The system requires an additional vessel where the fresh steam contacts the cream coming from the first deodorizing section. The steam then flows to the initial pasteurizing section. The procedure also permits the use of less steam or a reduction in cream flow rate. Modifications of this adaptation involving 3 and 5 stage units may be utilized for greater efficiency. Difficulty is sometimes encountered with overloaded condensers but can be partly alleviated by special procedures and inclusion of a surface condenser. In the improved deodorizing process, heat from steam flow and hot water can also be recovered for useful purposes, such as preheating cream, reducing overall steam consumption by approximately 50%. T. J. Claydon

DAIRY PLANT MANAGEMENT AND ECONOMICS

395. The dairy industry and the public. C. BATEMAN, Milk Ind. Foundation, Washington, D. C. Milk Dealer, 45, 6: 110. 1956.

Public relations usually means a planned effort to win public understanding of and support for an industry and its products. The dairy industry public relations problems may have their origin in large part in the following four areas: (1) The problem of surplus. (2) The problem of price and "spread." (3) The problem of regulation. (4) The problem of intra-industry relations. The approach to these problems is discussed. C. J. Babcock

396. Sales in 1956—up ten per cent. A. H. JOHNSON, J. W. Thompson Co., New York. Milk Plant Monthly, 45, 3: 16. 1956.

Consumer expenditures for food in 1955 climbed to approximately $69 billion or 35% above the $51 billion level five years ago. To keep up with our growing productivity and labor force, we must sell to consumers $26 billion more goods and services in 1956-57 than in 1955. This would mean a corresponding increase of 10% in the total potential market for dairy products if they succeed in holding their share of the food business. To keep up with our growing productive ability and probable population growth by 1960 the total market for food should be 25% greater than for the first quarter of 1955. C. J. Babcock

397. Reducing the error in tank milk measuring. H. E. CALBERT, Univ. of Wis., Madison. Milk Plant Monthly, 45, 3: 26. 1956.

One of the major problems in the bulk handling of milk has been to obtain an accurate volume measurement of the milk in the farm bulk tank. The most common causes of error are: (1) The farm bulk tank is not structurally stable. It will distort when filled or partially filled with milk. (2) The tank is not installed properly. It will not maintain a constant plane—preferably level. (3) Inaccurate calibration of the tank. (4) Inaccurate readings of milk measurements. Methods of overcoming these errors are discussed. C. J. Babcock

398. Vending developments. P. E. HORD. Milk Dealer, 45, 7: 85. 1956.

The 1955 sales of all types of automatically vended merchandise amounted to $1,700,000,000. Products were vended through about 3 million machines. Milk sales through about 25,000 machines amounted to about $40 million. This was an increase of about $18 million over 1954. Ice cream sales did not show as large an increase, but $23 million worth were sold through 22,500 machines in 1955. This was an increase of $2 million over 1954. C. J. Babcock

399. Installment financing of capital equipment. S. D. MADDOCK. Ice Cream Rev., 39, 10: 54. 1956.

The use of an industrial finance company to handle all major purchases is becoming a common practice, and purchases made from several different sources can be handled on a single contract by this procedure.

The more rapid depreciation schedules on equipment allowed under the present tax laws make installment purchasing of equipment highly desirable in many cases. Under this plan equipment can be paid for out of current depreciation reserve funds. The amount set aside for depreciation under the faster depreciation schedules should parallel the payments on installment financing.

The author points out that many firms have made a mistake in not taking advantage of the depreciation schedules now permitted by law.

W. J. Caulfield

400. Microfilm bookkeeping cuts billing cost. ANON. Milk Dealer, 45, 7: 38. 1956.

Microfilm bookkeeping saves $3,000 a year for Sinton Dairy, Colorado Springs, Colo., by eliminating the overtime formerly required when rendering monthly bills. The microfilm machine and reader are on a rental basis and cost $310 per year plus the films, which cost $14 per mo., for a total of about $500 per year. With photographic billing, clerk bookkeepers are not required and stationery costs are reduced ⅔. Details of the route sheets and monthly statements are given. C. J. Babcock

401. Cutting the accident rate. P. R. ELLSWORTH, Milk Ind. Foundation, Washington, D. C. Milk Plant Monthly, 45, 3: 29. 1956.

The dairy industry accident rate is the highest among food processing industries. Both lost-time accident rates and severity rates of accidents must be reduced if the industry is to enjoy better employee relations, less loss or product, less equipment damage, fewer "short" crews, plus lower industrial compensation and insurance premiums. Methods of lowering accident rates are discussed. C. J. Babcock

402. Delivery costs must be cut. T. D. LEATHERS. Milk Plant Monthly, 45, 3: 13. 1956.

Demands for higher wages by retail drivers may kill the retail milk business. Considering the present state of business, the human or personal element is about the only field open for any great reduction in dairy costs. In an ice cream plant a well organized production line and freezing schedule may help to reduce manufacturing costs. Labor costs in a plant hinge around the freezing room operations. An efficient production line usually means a low labor cost throughout the plant. Special deliveries send ice cream costs sky-rocketing. They are designed as an accommodation to the customer, but they can be eliminated. C. J. Babcock

403. The sensible attitude in Denver. T. D. LEATHERS. Milk Plant Monthly, 45, 4: 16. 1956.

In Denver there appears to be little evidence of price undercutting by either the milk plants or the supermarkets. The result has been a healthy sales picture on both retail and wholesale. In the last 4 years milk sales have increased 22% and the population, 18%. During 1955, 46% of the milk sales was home delivered and 54% was sold in stores. Quarts sell for 23¢ and ½ gal. for 45¢ in stores. Home-delivered milk costs 1¢ more per qt. and 2¢ additional per ½ gal. A base and reserve plan started by the local producers' association keeps production level. Promotional efforts by the milk plants, producers, and the local Dairy Council have increased sales and per capita consumption.

C. J. Babcock

404. "Weigh" to better profits. A. SANDERS. Milk Dealer, 45, 6: 42. 1956.

Accurate scales protect the producer, processor, and consumer. It is suggested that a scale be condemned if it has an error of more than 1½ oz. at 100 lb., 1 oz. at 75 lb., or 3/16 oz. at 5 lb. C. J. Babcock

405. Milk bottle container and carrier. G. ROBERTS. U. S. Patent 2,743,836. 1 claim. May 1, 1956. Offic. Gaz. U. S. Pat. Office, 706, 1: 106. 1956.

A box is described for holding bottles of milk between time of delivery and placing them in the home refrigerator. A carrier with a handle

is provided which fits into the box and which facilitates carrying the bottles.

R. Whitaker

FEEDS AND FEEDING

406. Winter feeding of dairy cows. I. The influence of level and source of protein and of the level of energy in the feed on milk yield and composition. W. Holmes, R. Waite, D. S. MacLusky, and J. N. Watson, Hannah Dairy Research Inst., Kirkhill, Ayr, Scotland. J. Dairy Research, **23**, 1: 1. 1956.

Twelve Ayrshire cows were used in four replicates of a 3 × 3 Latin square design feeding trial in which three levels of digestible crude protein (d.c.p.) and constant starch equivalents (s.e.) were fed one year and 3 levels of s.e. and constant protein were fed the next year. The levels of d.c.p. investigated were 10, 15, and 17% and the levels of s.e. utilized were 59, 67, and 75. The protein level had little effect on milk yield or composition. The higher s.e. resulted in a higher though not necessarily more economical production. The solids-not-fat in milk were increased when the s.e. was raised from 59 to 67 but no further increase was noted at the highest s.e. value.

It was advocated that those using the Woodman Standard should feed at 110-117% of the standard for total requirements to achieve optimal returns. J. D. Donker

407. Winter feeding of dairy cows. II. Dried grass produced in spring and autumn compared with other concentrates and with spring grazing. W. Holmes, Hannah Dairy Research Inst., Kirkhill, Ayr, Scotland. J. Dairy Research **23**, 1: 13. 1954.

Two groups of 12 Ayrshire cows were used in two feeding experiments which utilized a 3 × 3 Latin square design. Spring-dried grass (mainly rye) was compared to autumn-dried grass, and to a control concentrate which was different in the two trials. A home-grown concentrate with a starch equivalent of 58 and digestible crude protein of 12.5% was used in one trial and high-quality imported feeds (s.e. of 73.5 and d.c.p. 16.6%) in the second trial. The test rations were fed above a maintenance requirement in proportion to production on an equalized basis.

The spring-dried grass increased milk production 3-5% over the autumn grass but this was not significant. Home-grown grain was equal to spring grass for production response. The concentrate made up of imported feeds cause more milk flow than the grass concentrates. The milk production increase when cows were turned to pasture probably was due to a relative increase in nutrient intake.

J. D. Donker

408. Studies on Jula or Jowar (Andropogon sorghum) as cattle fodder. III. Hydrocyanic acid and phytic acid phosphorus content of different varieties of Jola fodders at different stages of growth. H. S. Lakke Gowda. Indian J. Dairy Sci., **8**: 47. 1955.

The hydrocyanic acid content of all varieties was maximum at the third week of growth and then decreased. Most of the acid was absent after 7 to 9 weeks. Phytic acid P was present in small quantities in the early stages of growth and increased gradually as the plant matured. The reverse was true of the case of total P.

W. C. van der Zant

409. Ruminant feed from cotton gin waste. A. Lent (assignor to Erly-Fat Livestock Feed Co.). U. S. Patent 2,744,824. 4 claims. May 8, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 2: 368. 1956.

A feed for ruminants consisting of 50-75% finely ground cotton gin waste and 50-25% molasses. R. Whitaker

GENETICS AND BREEDING

410. Variation in the chemical composition of milk with particular reference to the solids-not-fat. II. The effect of heredity. A. Robertson, R. Waite, and J. C. D. White, Inst. Animal Genetics, Edinburg, and Hannah Dairy Research Inst. Kirkhill, Ayr, Scotland. J. Dairy Research, **23**, 1: 82. 1956.

Five hundred pairs of daughter-dam comparisons of milk constituents collected from Ayrshire cows were used in correlation heritability estimates. Data were analyzed on a within-herd basis and were corrected to a standard age between pairs of animals. Correlations between values for daughters and dams were used to estimate heritability of the following constituents in milk: solids-not-fat, total solids, crude protein, casein, noncasein protein, fat, ash, and lactose. Heritability values ranged about a mean of 0.5 for solids-not-fat, crude protein, casein and ash, 0.35 for fat and lactose, 0.25 for yield and 0.13 for noncasein protein.

It was concluded from this work that selection for yield alone probably would not cause any great decline in solids-not-fat content of milk and that any decline could be counteracted by selecting for higher fat content of milk in addition to yield. J. D. Donker

411. Genetic improvement in milk yield in two herds of cattle and buffaloes in Egypt. A. A. Asker, M. T. Ragab, and S. A. Hilmy. Indian J. Dairy Sci., **8**: 39. 1955.

A study was made of the effect of selection for total milk, age at first calving, and calving interval. The records covered a period of 20 years for 177 cows and 175 buffaloes. There was no effective selection for age at first calving nor for calving interval for both cattle and buffaloes. Genetic improvement in total milk yield from selection of dams of heifers was 70 lb. with cattle and 133 lb. for buffaloes per generation. The annual genetic gain in milk yield was 18 lb., which amounted to 0.8% of the herd average. W. C. van der Zant

412. Heritability estimates and correlations of some economic traits in buffalo herd. O. P. AGARWALA. Indian J. Dairy Sci., **8**: 89. 1955.

Heritability estimates and phenotypic correlations between various economic traits were calculated for a herd of buffaloes over a period from 1934 to 1952. The heritability estimates for total butterfat in 305 days, % butterfat, and age at first calving were calculated as 6, 26, and 24%, respectively. The phenotypic correlations between various traits were statistically nonsignificant. Negative correlations were found between lb. of butterfat and weight at first calving, % butterfat and first calving, and age at first calving and mature weight.

W. C. van der Zant

HERD MANAGEMENT

413. Vacuum container milking system and apparatus therefor. G. R. DUNCAN (assignor to Zero Manufacturing Co.). U. S. Patent 2,739,568. 5 claims. Mar. 27, 1956. Offic. Gaz. U. S. Pat. Office, 704, 4: 718. 1956.

Milk from a vacuum type milker is delivered to an insulated milk-holding chamber equipped with a cooling coil and a motor-driven agitator.

R. Whitaker

414. Milking apparatus. L. J. SCHILLING (assignor to Schilling Electric Co.). U. S. Patent 2,740,422. 6 claims. April 3, 1956. Offic. Gaz. U. S. Pat. Office, 705, 1: 85. 1956.

A reservoir for milk coming from a milking machine which permits discharge of the milk through the operation of a specially designed float valve. R. Whitaker

415. Spray-cooled milk container and refrigerating system therefor. G. R. DUNCAN (assignor to Zero Manufacturing Co.). U. S. Patent 2,740,378. 10 claims. April 3, 1956. Offic. Gaz. U. S. Pat. Office, 705, 1: 75. 1956.

A combination cooler and storage vat in which milk from the cow is sprayed into the vat while it is under vacuum. R. Whitaker

416. Stall ararngement and vacuum container milking system. G. R. DUNCAN (assignor to Zero Manufacturing Co.). U. S. Patent 2,740,377. 10 claims. April 3, 1956. Offic. Gaz. U. S. Pat. Office, 705, 1: 74. 1956.

An arrangement for milking 4 cows at one time, in which the cows stand in close proximity and are milked with a pulsating vacuum type milker. The milk is conducted to a refrigerated storage reservoir. R. Whitaker

417. Tail sling. H. P. JOHNSON. U. S. Patent 2,740,380. 4 claims. April 3, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 1: 75. 1956.

A device for keeping a cow from swishing its tail, consisting of a sling attached to the tail which is flexibly connected to a noose placed around the cow's neck. R. Whitaker

418. Vacuum container milking system and apparatus therefor. G. R. DUNCAN (assignor to Zero Manufacturing Co.). U. S. Patent 2,742,876. 12 claims. Apr. 24, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 4: 745. 1956.

A combination of a bulk milk storage tank and refrigeration unit. Milk from a vacuum type milker is delivered under vacuum into the tank where it is cooled by contact with the side walls which are cooled by a spray of refrigerated water. Similar to Abstract #264, J. Dairy Sci. Abstr., **38**: A42. 1955. R. Whitaker

419. Adjustable milking machine cups. K. ROBEN (assignor to Westfalia Separator AG). U. S. Patent 2,744,496. 2 claims. May 8, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 2: 281. 1956.

A teat cup, designed to operate with a pulsating vacuum supply, has a rigid outer shell and flexible inner portion. The lip of the inner chamber is also flexible and so constructed that it can adjust to the size and shape of the individual teat to which it is applied.

R. Whitaker

420. How much to invest in a farm tank. C. E. FRENCH, Purdue Univ., Lafayette, Ind. Milk Plant Monthly, **45**, 3: 35. 1956.

A device called a "bulk tankulator" is illustrated. It is designed to answer how much one can afford to pay for a bulk tank. The bulk tank problem usually is approached in terms of savings per cwt. and initial investment. The savings vary among markets.

C. J. Babcock

ICE CREAM

421. One way shipping container for liquid mix. ANON. Ice Cream Rev., **39**, 10: 66. 1956.

A single service shipping container for liquid mix and similar products has been thoroughly tested by the Producers Creamery Co. at Springfield, Mo. More than 100,000 of the "Liquipak" containers were delivered to customers of the Producers Creamery in 1955 with entirely satisfactory results. Shipments were made as far as Fla. and Calif.

Numerous advantages are claimed for the Liqui-pak container, which consists of a seamless polyethylene liner and a water resistant outer carton. W. J. Caulfield

422. Five ways to reduce delivery costs of ice cream. R. A. WEIDNER, Pevely Dairy Col., St. Louis, Mo. Ice Cream Rev., **39**, 10: 64, 81. 1956.

Delivery costs, which have been steadily increasing, have not received as much attention as they deserve. Suggestions for reducing delivery costs of ice cream are discussed in detail and include: equalization of routes, reduced frequency and number of deliveries, use of call orders, elimination of special deliveries, and the use of prepared platform loading schedules.

W. J. Caulfield

423. Method for making frozen confections and apparatus therefor. C. K. NELSON (assignor to Eskimo Pie Corp.). U. S. Patent 2,739,545. 6 claims. Mar. 27, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 4: 711. 1956.

Ice cream and other frozen desserts are manufactured by extruding from a spout a continuous strip of stiff product, cutting individual sized portions, and hardening them by direct contact with a metal surface refrigerated to –30° F. or below. R. Whitaker

424. Refrigerating jacket for confection vending machine. R. G. TARR. U. S. Patent 2,742,268. 4 claims. Apr. 17, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 3: 588. 1956.

A method of refrigerating a bulk container of ice cream or other frozen confection inside a vending machine which dispenses portions of the product directly from the bulk container. R. Whitaker

425. Flow attachment for soft cream freezers. O. M. STALKUP. U. S. Patent 2,740,262. 7 claims. Apr. 3, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 1: 45. 1956.

To maintain soft-serve frozen desserts in condition for eating, a two-way dispensing valve is provided. If the product is too soft for serving, it is diverted through a heater into a pipe which connects with the mix inlet at the vat of the freezer. When the consistency is correct, the product is drawn by turning the valve. Introduction of fresh mix with the semi-frozen product is controlled by a thermostatic switch located in the mix pipe as it leaves the heating section. R. Whitaker

426. Implement for dispensing frozen comestibles. V. B. PHILLIPS, J. G. EVERETT, and R. P. HOEKEMA. U. S. Patent 2,740,365. 1 claim. April 3, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 1: 72. 1956.

A hand-operated ice cream dipper which delivers individual portions in the shape of squat cylinders. R. Whitaker

427. Continuous freezer. C. W. MERRILL. U. S. Patent 2,745,261. 11 claims. May 15, 1956. Offic. Gaz. U. S. Pat. Office, **706**, 3: 484. 1956.

A continuous ice cream freezer is described with special emphasis on the mode of application of the refrigerant to the freezing chamber. R. Whitaker

MILK AND CREAM

428. Teneur du yoghourt en quelque vitamins. (Amount of some vitamins in yoghurt). L. RANDOIN and J. CAUSERET. Le Lait, **36**: 129. 1956.

The amounts of thiamine, riboflavin, vitamin A, and carotene were determined on commercially and laboratory-prepared samples of yoghurt. In fresh yoghurt samples the B vitamins were present in amounts similar to that of fresh milk. Vitamin A activity in the commercially prepared yoghurt was below that of fresh milk because of the low butterfat content (0.8 to 1.7%). The stability of the various vitamins in yoghurt during storage was about the same as in milk. A. W. Rudnick, Jr.

429. L'inspection sanitaire du lait (Inspection of milk from the sanitary standpoint). G. THIEULIN and D. BOSILLE. Le Lait, **36**: 137. 1956.

The authors discuss in detail the advantage of using automatic temperature controls and the phosphatase test as means of determining adequacy of pasteurization or recontamination of pasteurized milk with raw milk.

A. W. Rudnick, Jr.

430. They put the lasso over off-flavors. W. A. DODGE and A. BRADFIELD, Univ. of Vermont, Burlington. Milk Plant Monthly, **45**, 4: 31. 1956.

An extensive program to improve the flavor of milk in Vermont is bringing results. Initially 70% of the milk marketed had undesirable flavors. Now only about 35% of the milk has off-flavors, and even better results are expected. This improvement in milk quality is the result of the application of sound production practices. C. J. Babcock

431. Studies on vitamin A in milk. VIII. Vitamin A in market milk. S. R. SAMPATH, C. P. ANATAKRISHNAN, and K. C. SEN. Indian J. Dairy Sci., **8**: 129. 1955.

A study was made of the variation in the vitamin A potency of market milk in Bangalore. The vitamin A potency of cow, buffalo, and mixed milk ranged from 336 to 1,025, 492 to 1,536, and 449 to 1,200 I.U./lb, respectively.

W. C. van der Zant

432. Studies in physico-chemical properties of milk. V. Viscosity of milk. B. R. PURI and H. L. GUPTA. Indian J. Dairy Sci., **8**: 78. 1955.

The viscosity of milk as determined with the Ostwald viscosimeter varied slightly with the capillary width of the instrument. Fairly consistent values for the viscosity were obtained with viscosimeters of a fairly wide range of capillary size (time of flow of 20 ml. of water 28 to 45 sec. at 30° C.). Storage of milk at 25° C. for 10 hr., at 30° C. for 6 hr., and at 35° C. for 3 hr. did not affect its viscosity. The temperature coefficient of viscosity of milk remained almost the same as addition of water up to the extent of 35%. Heating milk at 80° C. for 5 min. slightly increased the viscosity. Proteins contributed more towards the viscosity than any other major constituent of milk.

W. C. van der Zant

433. Studies in physico-chemical properties of milk. VI. Viscosity of milk on the addition of ammonia. B. R. PURI and H. L. GUPTA. Indian J. Dairy Sci., **8**: 100. 1955.

An increase in the viscosity of milk was observed on addition of ammonia. The viscosity of this mixture varied with a change in the protein content of the milk. An empirical formula connecting viscosity with the protein content was established. W. C. van der Zant

434. Application of circular paper chromatography method for the detection of adulteration of milk. B. V. RAMACHANDRA, N. N. DASTUR, and K. V. GIRI. Indian J. Dairy Sci., **8**: 83. 1955.

To mask adulteration of milk with water cane sugar frequently is added. A single chromatographic procedure is described to detect adulteration of milk with sugars other than lactose. W. C. van der Zant

435. Het opsporen van nabesmetting by de bereiding van gepasteuriseerde melk (Detection of post-pasteurization infection of milk). TH. E. GALESLOOT. Neth. Milk Dairy J., **9**: 227. 1955.

An indication of post-pasteurization contamination was obtained by determining the increase of the plate count after storing the pasteurized milk at 15° C. for 24 hr. This test gave an indication of recontamination with gram-neg., nonsporeforming bacteria, which were mostly psychrophilic in nature. Non-thermodurie streptococci were detected by the increase in acidity after storing the pasteurized milk at 20° or 27° C. for 24 hr. W. C. van der Zant

436. De invloed van de pasteurisatie temperatuur op de duurzaamheid van gepasteuriseerde milk (The influence of the heat treatment on the keeping quality of pasteurized milk). TH. E. GALESLOOT. Neth. Milk Dairy J., **9**: 237. 1955.

Samples of milk were heated for 5 min. at 63°, 85°, and 100° C. After cooling, the samples were inoculated with bacteria usually responsible for the deterioration of commercially HTST milk. The organisms used were *S. lactis, S. cremoris, L. dextranicum, A. viscosum, Ps. putrefaciens, Ps. nigrifaciens, Ps. fluorescens, Ps. viscosa, E. coli,* and *A. aerogenes.* After inoculation the samples were stored at 15° or 20° C. for 24 or 16 hr. and the extent of their growth was determined by making plate counts. The initial development of the lactic acid bacteria, and some of the *Pseudomonas* species in milk heated at 63° C. was inhibited. The development of some other *Pseudomonas* species, coliform bacteria and achromobacteria was only very slightly inhibited. No differences in growth were observed in the milks at the higher temperatures. W. C. van der Zant

437. Vitamin D in Milk. C. ENGEL. Neth. Milk Dairy J., **9**: 139. 1955.

A summary of vitamin D assays of condensed irradiated milk is presented. Milk collected during November was condensed to half its original volume (7.55% fat) and irradiated according to the Scholl-Scheer Steinheil process. The vitamin D content ranged from 160 to 200 I.U. per l. According to the author, Wagner's claim (Milchwissenschaft, **8**: 379. 1953) that U. V. irradiation of milk is not effective in improving its vitamin D content has been disproved. W. C. van der Zant

438. The condition of calcium phosphate in milk. N. EVENHUIS and TH. R. DE VRIES. Neth. Milk Dairy J., **9**: 146. 1955.

The precipitation of calcium phosphate on heating (100-115° C. 5-25 min.) was studied. Yeast cells did not accept calcium phosphate at low temperatures. The precipitation of calcium phosphate on yeast cells was a crystallization and/or recrystallization process. Yeast cells did not have a specific action since cotton wool and other substances with a large surface area could be used. The rate of crystallization of calcium phosphate on surface areas depended to a great extent on the presence of calcium phosphate centers. Preheated milk gives rise to less formation of calcium phosphate centers and calcium phosphate deposition on surface areas than raw milk. W. C. van der Zant

439. Individual cream carton. F. L. CARTER. U. S. Patent 2,740,573. 1 claim. April 3, 1956. Offic. Gaz. U. S. Pat. Office, **705**, 1: 125. 1956.

A disposable carton for individual size portions of cream is described. A tab is provided which is attached to a tear line for easy opening of the container to expose a pouring spout.
R. Whitaker

440. Flavor defects: cause and cure. ANON. Milk Plant Monthly, **45**, 3: 22. 1956.

Leading dairymen discuss their flavor problems and summarize the ways to prevent off-flavors. Close supervision on the farm, proper processing, and additional research to learn more about the many factors involved in the development of rancidity are recommended.
C. J. Babcock

MILK SECRETION

441. A radiographic method for the study of the function of the cow's udder. J. BILEK and M. JANKOVSKY. Nature, **177**, 4508: 582. 1956.

A cow was acclimatized to being milked in an X-ray room for 10 days. A test was conducted by inserting a sterile "Nelaton" No. 2 rubber catheter dipped in a urological antiseptic lubricant into the teat. About 20 ml. of milk was removed and replaced with 20 ml. of a contrast medium, consisting of 35% "Ultraren" (aqueous iodide solution) at 37° C. This yielded a satisfactory X-ray picture. The distal portion of the cistern was seen to change in a few seconds from a smooth cylindrical shape to an hour-

glass shape when the teat was squeezed as in milking. Contractions were periodic, at 1 or 2 per min. and lasted for about 15 min. Intravenous injection of oxytocin caused milk ejection but no contractions were noted.

R. Whitaker

442. Variation in the chemical composition of milk with particular reference to the solids-not-fat. I. The effect of stage of lactation, season of year, and age of cow. R. Waite, J. C. D. White, and A. Robertson, Hannah Dairy Research Inst., Kirkhill, Ayr, and Inst. of Animal Genetics, Edinburg, Scotland. J. Dairy Research, **23**, 1: 65. 1956.

The milk from 814 Ayrshire cows was sampled 6 times during a lactation and analyzed for total solids (T.S.), solids-not-fat (S.N.F.) by difference, crude protein (C.P.), casein, lactose, and fat. Lactation and season effects in the analytical data were separated statistically. The av. of the 6 samples was used to arrive at a lactation value which was used to determine age effect on milk composition.

Milk yield rose to a peak 45 days after calving and then declined to the end of lactation. T.S., S.N.F., fat, C.P., and casein decreased rapidly for the first 45 days, and the fat and T.S. continued to decrease for another month before these constituents increased throughout the remainder of the lactation. Lactose showed an opposite trend to fat and protein throughout lactation. Lactose in young cow's milk did not drop as much as in milk from older cows. Seasonal effects were not as marked as lactation effects but were definite. Yields increased from Jan. through June and then fell to a minimum in Oct.-Nov. Fat content was the inverse of yield. The protein constituents were highest in May-June and Sept., and lowest in late winter. Lactose content generally followed the yield curve. T.S. and S.N.F. were lowest in Mar. and Apr., and S.N.F. were highest in May-June, and T.S. were highest in Oct.

As age of animals increased, milk yield increased through 5 years and the composition became less rich in S.N.F. with age.

J. D. Donker

443. Variation in the carotene and vitamin A contents of herd butterfats throughout lactation. A. K. R. McDowell, Dairy Research Inst., Palmerston North, New Zealand. J. Dairy Research, **23**, 1: 111. 1956.

Butterfat collected on a herd basis from an autumn calving herd, a spring-calving herd, and from a butter factory was analyzed for carotene and vitamin A. The carotene content of butter from all three sources reached a maximum in the spring and was lowest in summer. In certain years autumn butter showed a material increase over summer butter. These changes appeared to be related to availability of carotene in the forage. The vitamin A content was relatively uniform throughout the year, probably due to storage of vitamin A in the liver and the fact that carotene in the forage never became deficient. The rises in vitamin A levels which were seen in spring-calved cows in autumn was the result of seasonal conditions and not a lactational effect. The inclusion of early post-colostral fat increased carotene and vitamin A in butter samples in August above what would be expected from seasonal effects. It was concluded that the fat in the recently-fresh cow contains higher than "normal" values for carotene and vitamin A for 10-12 days after calving.

J. D. Donker

444. Influence of frequency of milking on milk production. O. P. Agarwala and D. Sundaresan. Indian J. Dairy Sci., **8**: 94. 1955.

Six cows with equally balanced udders in an early stage of lactation were used. The 100-day test period was divided in 4 equal periods. In each period one half of the udder was milked 3× daily, and the other half 2×. The production of the halves milked 3× daily exceeded those milked twice a day by 8.4%. The av. production of the cows was 14 lb. per day.

W. C. van der Zant

NUTRITIVE VALUE OF DAIRY PRODUCTS

445. Amino acid balance in nutrition. C. A. Elvehjem, Univ. of Wis., Madison. J. Am. Dietetic Assoc., 32, 4: 305. 1956.

An excess of protein in the diet may be deleterious when that protein is unbalanced with respect to amino acids, as are many of the plant proteins. In contrast to these plant proteins, a balanced protein such as casein can be taken in excess without harm.

F. E. Rice

446. The prophylaxis of allergic disease with special reference to the newborn infant. J. Glaser, New York J. Med., 55: 2599. 1955. Abs. in Am. J. Dis. Children, 91, 4: 404. 1956.

To avoid the possibility of milk allergy in the newborn, author suggests that pergnant mothers drink only a pint of boiled milk each day and eat no cheese. It is the author's contention that an allergy from milk (and other foods) can be transmitted to the offspring through the food consumed by the mother.

F. E. Rice

447. Renal function of the newborn: at term and premature. P. Royer. Etudes neo-natal. 3: 177 (Sept.) 1954. Abstract in Am. J. Dis. Child. 91, 3: 295. 1956.

Kidney functions of the newborn are imperfect and not complete until 6 months to 2 years of age. Cow's milk without added carbohydrate, fed in amounts sufficient to provide calorie needs, carries amounts of salts and proteins too large for the excreting power of the kidney. On a human milk diet, with lower protein and mineral content, there is less difficulty. Edema may ensue, as may an increase in weight, and sometimes fever, called "powdered milk fever." An increased water intake will tend to induce a cessation of these symptoms.

F. E. Rice

DIFCO

Bacto-Dehydrated Media for Microbiological Assay of

VITAMINS and AMINO ACIDS

These media contain all the necessary nutriments for the growth of specified test organisms for the microbiological assay of vitamins and amino acids except for the component under assay. These basal media require only the addition of specified increasing amounts of the vitamin or amino acid being assayed to obtain a growth response which may be measured by acidimetric or turbidimetric methods for the construction of standard curves. The vitamin or amino acid content of the material under assay is determined by adding appropriate concentrations of the test substance to the basal medium and comparing the growth response obtained with that of the standard.

BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM
BACTO-CYSTINE ASSAY MEDIUM

The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-B_{12} CULTURE AGAR USP
BACTO-B_{12} INOCULUM BROTH USP
BACTO-NEUROSPORA CULTURE AGAR

BACTO-VITAMIN FREE CASAMINO ACIDS, dehydrated, is an acid hydrolysate of vitamin free casein prepared especially for laboratories investigating microbiological assay of vitamins.

Descriptive literature available upon request

DIFCO LABORATORIES
DETROIT 1, MICHIGAN